QUALITY IMPROVEMENT OF ‘PHULAE’ PINEAPPLE PUREE BY HIGH PRESSURE PROCESSING

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ABSTRACT

'Phulae' pineapple (*Ananas comosus* L. Merr) is a popular commodity and a Geographical Indication of Chiang Rai province, Thailand which is cultivated all year and typically consumed fresh. The oversupply and mismanaged postharvest of the crops leading to the lower price of the product. The aim of the study was to study the effect of high-pressure processing (HPP) on the quality improvement of 'Phulae' pineapple puree to be a value added for the product. The study was divided to 4 parts. Part I: preliminary study to select the suitable condition for the HPP, Part II: to study the effect of HPP during storage, Part III: to study the effect of HPP on Glycemic Index (GI) and dietary fiber, and Part IV: to study the effect of HPP on the anti-inflammatory activity.

For the preliminary, 'Phulae' pineapple puree was prepared fresh and treated with HPP (400 and 600 MPa for 5, 10, and 15 mins) as well as heat treatment (HT) (80°C for 10 mins). The preliminary study shows that HPP could retain all of the quality attributes of 'Phulae' pineapple puree, producing higher antioxidant activity and bioactive compound in the product, with the same amount of microbial reduction as heat treated sample. HPP at 400 and 600 MPa for 10 mins were used for the further experiment alongside the fresh and HT sample, due to its high amount of antioxidant capacity and bioactive compound.

During storage, the physiochemical attributes such as pH, TSS, and TA were stable but the color are slightly change. Despite the similar microbial safety during the storage with the HT sample, all the bioactive compounds and antioxidant activity of the samples were decreasing overtime during the storage.

The study also found that GI of all samples is between 40.36 to 44.47, with HPP product showed the higher GI, due to the change of the sucrose to glucose during the treatment. However, the number for all samples are <55 which still considered as low GI food. The study also found out that HPP product have higher anti-inflammatory effect compared to fresh product. However, the amount reduced during storage. HPP treated sample at 400 MPa for 10 mins is the most optimal treatment for 'Phulae' pineapple puree due to its lower energy consumption (lower pressure) but similar result with the HPP treatment at 600 MPa for 10 mins.

Keywords: High Pressure Processing, 'Phulae' Pineapple Puree, Quality Attributes, Functional Properties

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ABBREVIATIONS AND SYMBOLS

°C	Degree Celcius
a*	Red-green
APC	Aerobic Plate Count
b*	Yellow-blue
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
GI	Glycemic Index
HHP	High Hydrostatic Pressure
HPP	High Pressure processing
HT	Heat Treatment
L*	Lightness
NO	Nitric Oxide
PPO	Polyphenol Oxidase
TE	Trolox Equivalent
TPC	Total Phenolic Compound
YM	Yeast and Mold Count

CHAPTER 1

INTRODUCTION

1.1 Background

Nowadays, more people are looking after their wellness. It defined by few different aspects, not only for their better mind, health, fitness, sleep, appearance and body, but also for better nutrition (Callaghan et al., 2021). The better nutrition defines how food could help them reach their wellness goal, which include the tracking of nutrition on each food and shifting to healthier food options. Fruits and vegetables are some options that usually preferred as heathy food. Their popularity is so enormous, that people not only like to consume them on their fresh form but also the processed product, such as in jam, juice, and puree.

Processed product of fruit and vegetable are very convenient to consume and has longer shelf life compared to its fresh form. However, during the processing, usually it involved thermal-processed which could affect the nutrition content, sensory, and the appearance of the product. To avoid this, various novel-non thermal processing should be applied, such as high pressure processing (HPP), pulse electric field (PEF), ultrasound, pulse light (PL), and irradiation. Among all of the processing method mentioned, HPP is one of the most successful non-thermal processing which only use minimal heat but could extent the product shelf life by killing the food pathogen, and maintain the sensory properties and nutritional value of the product by applying the high pressure (Huang et al., 2017; Aber, 2019). Some studies also shown that it could increase the antioxidant activity and retain some bioactive compound in the fruit and vegetable-based product. This process not only could kill microorganism including bacteria, yeast and mold but also deactivate the enzyme (such as peroxidase and polyphenol oxidase) and chemical reaction within the product.

Pineapple is widely grown in Thailand. It is a high nutrition fruit with high demand by consumer due its distinctive characteristic (taste, flavor, juiciness) among the tropical fruit (Uddin et al., 2019). It is high in vitamin C, minerals, water and crude

fiber. Thailand is top ten country which has the highest pineapple export and production (FAO, 2021). Known as one of the Geographical Indication (GI) of Chiang Rai province, Thailand, 'Phulae' pineapple (*Ananas comosus* L. Merr), a sub-variety of Queen group pineapple are widely studied in the recent year. It has a small round shape with crunchy, aromatic, light-yellow color pulp and sweet taste (Kongsuwan et al., 2009). This pineapple variety is cultivated all year round and usually consumed fresh. However, due to the oversupply in various season and the mismanaged postharvest, it could lead into lower price of the product (Chuensombat et al., 2019). To overcome this problem, further processing step could be an alternative option. 'Phulae' pineapple could be use as raw material to produce a puree product. The processing step not only preserve the nutrition of the product, but also a value added to the product. Thus, the development of product using 'Phulae' pineapple would be beneficial, not only for the consumer, but also the pineapple producer especially in Chiang Rai region.

High pressure processing is applied in many of pineapple product range. The HPP processed not only been done to the fresh-cut pineapple product (ALEMÁN et al., 1994), but also the processed product like pineapple compote (Uddin et al., 2019), pineapple juice (Wu et al., 2021), pineapple concentrate (Khalid et al., 2016) and pineapple puree (Chakraborty et al., 2016). Despite the various recent study of the effect of HPP in pineapple product, including in the puree form, there is none of the study that focused on the application of HPP in 'Phulae' pineapple puree. This cultivar contains special aromatic compound which is its unique characteristic and need to be maintained by non-thermal processing. The application of HPP could be an alternative technology to add value of 'Phulae' pineapple product. Therefore, the aim of this study was to investigate the effect of HPP treatment on the quality improvement (physiochemical and microbial), bioactive compound, glycemic index, and anti-inflammatory activity of 'Phulae' pineapple puree.

1.2 Objectives

1.2.1 To determine the most suitable process of ‘Phulae’ pineapple puree production using HPP treatment by comparing it with HT treatment.

1.2.2 To study the effect of HPP treatment on the quality attributes (physiochemical, microbial, and bioactive compounds), dietary fiber, glycemic index, and anti-inflammatory activity of ‘Phulae’ pineapple puree.

1.3 Scope of Research

The experiment to study the effect of HPP in ‘Phulae’ pineapple puree was divided into four different parts as followed.

1.3.1 Effect of HPP on Quality Attributes, Bioactive Compounds, and Antioxidant Activity of ‘Phulae’ Pineapple Puree (Preliminary)

‘Phulae’ pineapple puree was treated with HPP treatment at 400 and 600 MPa for 5, 10 and 15 mins. Before being analyzed, the sample were stored at 5±1°C in a dark condition. The quality attributes, bioactive compounds and antioxidant activity that evaluated were as followed.

1.3.1.1 Physiochemical attributes, such as color (L* and b*), total soluble solids (TSS), pH, and titratable acidity (TA) were evaluated.

1.3.1.2 Microbial attributes, such as aerobic plate count (APC) and yeast and mold (YM) were evaluated.

1.3.1.3 Bioactive compounds, total phenolic compound (TPC) and vitamin C content were evaluated.

1.3.1.4 Antioxidant activities, including 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity assay (DPPH) and ferric reducing antioxidant power (FRAP) assay were evaluated.

This part was also a screening process for the next part of the study. Two optimal condition which fulfill the best criteria (lowest microbial count, higher vitamin C retention, bioactive compounds, and antioxidant activity) were examined in the next part of the study and compared with the fresh sample and control (heat treated sample).

1.3.2 Effect of HPP on 'Phulae' Pineapple Puree's Quality Attribute, Bioactive Compounds and Antioxidant Activity During Storage

The quality attributes, bioactive compounds, and antioxidant activity of 'Phulae' pineapple puree which selected after the first part of the study were kept for 8 weeks (stored at 5±1°C in a dark condition) and were evaluated weekly as followed.

1.3.2.1 Physiochemical attributes, such as color (L* and b*), total soluble solids (TSS), pH, and titratable acidity (TA) were evaluated.

1.3.2.2 Microbial attributes, such as aerobic plate count (APC) and yeast and mold (YM) were evaluated.

1.3.2.3 Bioactive compounds, total phenolic compound (TPC), and vitamin C content were evaluated.

1.3.2.4 Antioxidant activities, including 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity assay (DPPH) and ferric reducing antioxidant power (FRAP) assay were evaluated.

1.3.3 Effect of HPP on 'Phulae' Pineapple Puree's Glycemic Index

'Phulae' pineapple puree which selected after the first part of the study were evaluated using *in vitro* digestion model.

1.3.4 Effect of HPP on 'Phulae' Pineapple Puree's Anti–Inflammatory Activity

'Phulae' pineapple puree which selected after the first part of the study were evaluated. The nitric oxide radical scavenging activity were evaluated.

1.4 Expected Benefit

1.4.1 Expected Outputs

1.4.1.1 To obtain the suitable condition to produce and maintain quality of ‘Phulae’ pineapple puree using HPP.

1.4.1.2 To obtain the suitable condition of HPP to improve the functional properties of ‘Phulae’ pineapple puree which enhance the bioactive compound, antioxidant, and anti-inflammatory activity.

1.4.1.3 To obtain the suitable condition of HPP for reducing the glycemic index in ‘Phulae’ pineapple puree.

1.4.2 Expected Outcomes

1.4.2.1 The application of HPP could be an alternative technology for consumer to obtain healthier functional food which impact on better health condition.

1.4.2.2 The application of HPP could be used in another fruit puree product and applied by the industry.

1.4.2.3 The information obtained from this study could be used for further research related to HPP and anti-inflammatory activity.

CHAPTER 2

LITERATURE REVIEW

2.1 Pineapple

Pineapple (*Ananas comosus*) is an edible member or the family Bromeliaceae, which is grown in several tropical and subtropical area. There is uncertainty of the origin of pineapple but Parana-Paraguay Basin considered as the possible area (FAO, 2021). These days, pineapples are produced in a lot of part of the world. Among all of those, the major pineapple exporter in the world market are Philippines, Thailand, Costa Rica, Indonesia, Chile, Ivory Coast and South Africa (Hossain, 2016).

Pineapple is a perennial crop which could live for almost 2 years. Even if it could be available all year round, the temperature and humidity paling a huge role that affect the fruit quality, or the acid and sugar content (FAO, 2021). The combination of optimum temperature (22 to 26°C) and high relative humidity will produce juicy fruit with low acid content (FAO, 2021)

Pineapple is rich in macro micronutrient. It contains abundant amount of calcium, potassium, vitamin C, carbohydrates, crude fiber, water and several minerals that could be beneficial for digestive system and helps in maintaining ideal weight and balanced nutrition (Hossain, 2015). The micronutrient, especially mineral and vitamins are important for human diet (Hounhouigan et al., 2014). The details of the amount of each macro and micro nutrients are presented on table 2.1.

As the third most commercial important tropical fruit, high quality pineapple with rich nutrition and special flavor are highly favored by customer worldwide (Sun et al., 2016). Pineapple could be used or consumed as fresh (FAO, 2021) or processed product, like pineapple juice (Hossain, 2016), dried sliced pineapple (Marcel et al., 2014), spray dried pineapple powder (Wong et al., 2015), and pineapple puree (Chutintrasri & Noomhorm, 2007).

Table 2.1 Nutrients in 100 grams of pineapple

Nutrients	Amount
Energy	52 calories
Dietary fibre	1.40 g
Carbohydrate	13.7 g
Protein	0.54 g
Iron	0.28 mg
Magnesium	12 mg
Calcium	16 mg
Potassium	150 mg
Phosphorus	11 mg
Zinc	0.10 mg
Vitamin A	130 I.U
Vitamin B 1	0.079 mg
Vitamin B2	0.031 mg
Vitamin B 3	0.489 mg
Vitamin B 6	0.110 mg
Vitamin C	24 mg

Source Hossain (2015)

2.1.1 Pineapple Production

Pineapple is one of the major tropical fruits with the highest export quantity in the world beside mango, mangosteen, guava, banana, papaya and avocado (FAO, 2021). The production of pineapple globally is around 27.82 million metric tons in 2020 and estimated to grow 2% annually and reach 37 million metric tons by 2030. OECD-FAO (2021) expected 40% of the global pineapple production remains in Asian region which is the largest among the other region in the world. Despite Asia is being the largest producer globally, with Philippines as the largest global producer, Costa Rica, who is the second largest global producer is the one who accountable for 70% of global shipment (export). This indicate that the pineapple production in Asia is mostly consumed locally.

Thailand is one of the top ten country (9th) in the world with the highest pineapple production which accountable for around 1.26 million metric tons in 2023 (Statista, 2025). The total production value over 500 million USD (FAO, 2020). There are 3 major group of pineapple that produce in Thailand which are Queen, Cayenne, and Spanish (Popluechai et al., 2007). In total, there are more than 25 species (cultivar) of pineapple cultivated in Thailand, such as 'Phuket', 'Phulae', 'Nanglae', 'Intrachitdang', 'Intrachitkow', 'Pattavia', 'Tradsithong', 'Sawee' and 'Petburi'. Pineapple is cultivated all over Thailand, but each region has different cultivar produced.

Chiang Rai province, which is the north region of Thailand has 3 major pineapple cultivars, such as 'Pattawai', 'Phulae', and 'Nanglae'. The total production of the pineapple in Chiang Rai province is almost 150 tonnes with the highest production of 'Pattawai' (56%), follow by 'Phulae' and 'Nanglae', 40% and 4% respectively (Chiang Rai Agricultural Extension Office, 2021). Compared to 2020, in 2021 the production of 'Phulae' pineapple rose by almost 10%, contradict with the lower production of 'Pattawai' and 'Nanglae' in the same year.

2.1.2 'Phulae' Pineapple and Its Product

'Phulae' pineapple (Figure 2.1) is one of the Queen group pineapples that cultivated in Chiang Rai province, with Bandu, Thasud, and Nanglae sub-district as the main producing area. It is a geographic indication of Chiang Rai province. The fruit are small with round tout shape and weight around 150-1000 grams with green or greenish yellow skin when it reaches the ripening stage. It has relatively light-yellow color with crispy texture and tend to be very aromatic (Kongsuwan et al., 2009). 'Phulae' pineapple has sugary, sweet, honey, and ripe fruit aroma and flavour (Sirimuangmoon, 2021).

Source Tangjitrapitak (2022)

Figure 2.1 ‘Phulae’ Pineapple

Compared to ‘Nanglae’ pineapple, ‘Phulae’ pineapple has higher vitamin C, total phenolics, and β-carotene, as shown in table 2.2 (Kongsuwan et al., 2009). It is also more preferred by consumer in term of texture and appearance. Study by Sirimuangmoon (2021) showed that ‘Phulae’ pineapple has higher overall liking score compared to ‘Nanglae’ variant.

Table 2.2 ‘Phulae’ and ‘Nanglae’ pineapple vitamin C, total phenolic, and β-carotene content

Content	‘Phulae’	‘Nanglae'
Vitamin C (mg/100g FW)	18.88	6.45
Total Phenolics (mg GAE/100g FW)	26.20	20.28
β-carotene (µg/100 g FW)	3.35	1.41

Source Kongsuwan et al. (2009)

‘Phulae’ pineapple are mostly consumed fresh, sell in its original form. However, due to its popularity and high demand, nowadays we could find several products that use ‘Phulae’ pineapple as raw material. Recently in Thailand, we could find it in the form of juice, yoghurt, or dehydrated product (Figure 2.2).

Source D-Fresh Phulae (2022), Dehydrated Phulae Slices (2022), Meiji Live Phulae Pineapple Flavoured Drinking Yoghurt (2021), Café Amazon (2020)

Figure 2.2 Range of 'Phulae' pineapple product

2.1.3 Pineapple Puree

Pineapple puree could be made by peeling the pineapple, rinse, cut, and chopped it into a puree (Diamante et al., 2014). Sometimes pineapple puree is mistakenly identified as pineapple juice. However, their processing method is slightly different. In pineapple juice there is filtration or extraction step in which make the juice look clearer (Carvalho & Silva, 2010). It sometimes uses blanching before the extraction process (Maia et al., 2007). Some method also uses an enzyme treatment in order to clarify the pineapple juice (Carneiro et al., 2002). Like some other juice, pineapple juices are usually pre-pasteurized and then aseptically filled (*Canning of Juices, Fruit Drinks, and Water*, 2016),

The commercial pineapple puree is packed and sterilized or freeze to prolonged the shelf life. Pineapple puree is highly used in the industry for producing fruit filling, pineapple juice mixture, pineapple jam, and baby food (Uan-On & Senge, 2008). It also commercially advertised to use as main ingredient to make some food (e.g. ice cream, gelato, sorbet, frozen desserts, yoghurt, sauces, preserves) and beverages (e.g. homebrewing, microbrewing, nano brewing, craft brewing, fruited beers, fruited sours,

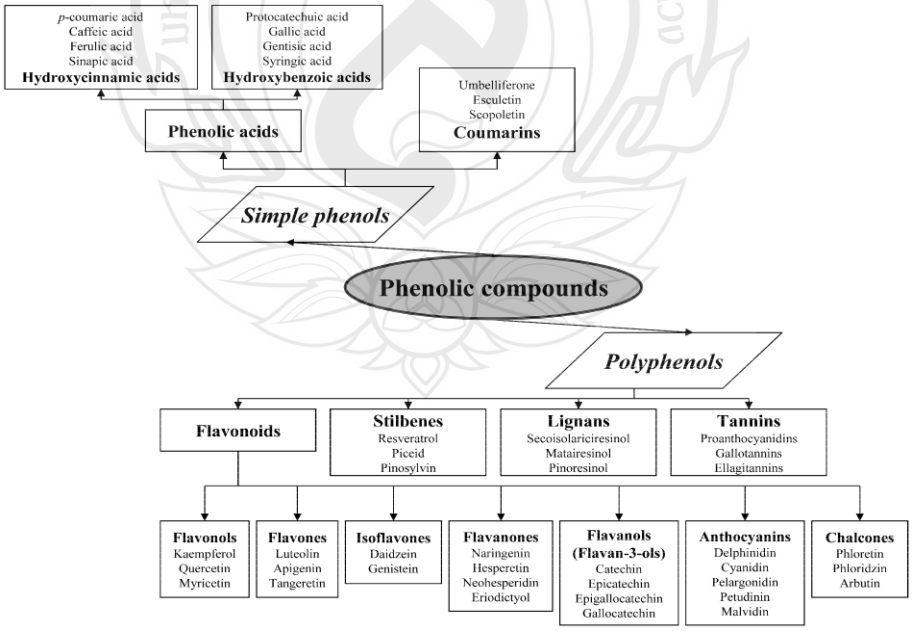
cocktails, juices, seltzer, ciders, kombucha, mead, tea) (*Real Pineapple Puree | 100% Natural Fruit*, 2021).

Pineapple puree are easily got non-enzymatic browning reaction during the thermal processing. A study found that the lightness is the most sensitive measure of color change during the pineapple puree thermal processing (Chutintrasri & Noomhorm, 2007).

2.2 Bioactive Compound, Glycemic Index and Anti-inflammatory Activity in Pineapple Puree

2.2.1 Phenolic Compounds

Phenolic compounds (Figure 2.3) are one of the secondary metabolites in plant which produced by the phenylpropanoid metabolization involving shikimic acid and pentose phosphate. One of the main characteristics of the phenolic compound are the benzene rings with one or more hydroxyl substitute (Lin et al., 2016). The phenolic compounds could be varied, from simple phenols to complex polyphenols compound (Soto et al., 2015). Phenolic compounds also have a lot of function in plant, depends its



Source Soto et al. (2015)

Figure 2.3 Phenolic compounds

characteristic. Generally, it acts as antioxidants, structural polymers (lignin), attractants (flavonoids and carotenoids), UV screens (flavonoids), signal compounds (salicylic acid, flavonoids) and defense response chemicals (tannins, phytoalexins) (Lin et al., 2016). In human, phenolic compounds could be beneficial as anti-aging, anti-inflammatory, anti-proliferative activities, and antioxidant. Consuming rich-antioxidant food will reduce the risk or certain chronic disease such as diabetes, cancer, and cardiovascular disease by the management of oxidative stress (Lin et al., 2016).

The major phenolic compound found in pineapple are polyphenol such as flavonoid and tannins (Pajarito et al., 2017). Those results indicate that pineapple is a potential source of phytochemical with antioxidant activity (Pajarito et al., 2017). The phenolic compound in pineapple can be vary, as seen in table 2.3. It depends on how do we cultivate the plant (Yapo, 2011) and the ripening stage of the pineapple (Ding & Syazwani, 2015)

Table 2.3 Phenolic compound in pineapple

Source	Total phenolic (mg GAE/ 100 g DW)	Total Flavonoid (mg CE/ 100 g DW)	Tannin (mg tannin eq./ 100 g DW)	Phenolic Compound
(Pajarito et al., 2017).	160.7 - 197.4	7.7 - 14.7	77.1 - 124.2	-
(Yapo, 2011)	-	-	-	gallic acid, gentisic acid, syringic acid, vanillin, ferulic acid, sinapic acid, isoferulic acid and o-coumaric acid
(Rasheed et al., 2012)	117.75	-	-	-

2.2.3 Glycemic Index and Dietary Fiber

Glycemic index (GI) known as a system that classify carbohydrate containing food based on how fast the food are digested and absorbed during the postprandial period. It measures the quality of carbohydrate's quality based on the direct effect on the blood glucose level during 2 hours after meal.

The differences of the GI range of the sample are more likely due to the biological material used (e.g. cultivar, maturity) or the preparation method (Elizondo-Montemayor et al., 2015). The processing, cooking and storage method also contribute to the variety of GI's value. Another factor that contributes to the different GI from the same fruit is the diverse condition (form) of the fruit itself, whether it's the whole fruit, puree, or juice (Foster-Powell et al., 2002). High consumption of food with high GI is associated with an increased risk of developing type 2 diabetes, cardiovascular disease, and certain cancer (Foster-Powell et al., 2002).

Dietary fiber (DF) is the non-digestible type of carbohydrates and lignin which is not degraded in upper gut in human digestive system. There are 2 types of DF, based on its solubility in water, which is the soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). SDF are viscous and highly fermentable while IDF are the opposites. However, both types could reduce the inflammation, energy density and weight gain while increase bulking effect and satiety in human (Weickert & Pfeiffer, 2018).

Low-GI diet is usually high fiber diet, which means it contains high dietary fiber. However, different type of DF will have different response to GI. Soluble dietary fiber could reduce the postprandial glucose response (GI) due to its viscous form and gel-forming in which delaying the absorption of dietary carbohydrate (Weickert & Pfeiffer, 2018).

Pineapple itself has moderate glycemic index which is 59 (Defeat Diabetes Foundation, 2018). However, another study found that pineapple pulp could have glycemic index as high as 93 in the fruit and 96 in the core, compare to bread with 100 glycemic index which is count as high (Cordenunsi et al., 2010). A lot of study has been found that pineapple is rich in dietary fiber, especially on its core (Prakongpan et al.,

2002) and peels (Larrauri et al., 1997). The total dietary fiber of pineapple is 3.9% on dry basis, with IDF (2.3%) content higher than SDF (1.82%) (Velderrain-Rodriguez et al., 2016).

2.2.4 Anti-inflammatory Activity

Inflammatory response is body's natural defense mechanism which triggered by noxious stimuli (Ke et al., 2021). Human body will be protected from injuries to tissue and microbial invasion with a proper inflammatory response. Furthermore, the tissue and cell stability will be increase, alongside the immune system (Tung et al., 2008). During inflammation, the body will activate several immune cell to produce inflammation mediator such as nitric oxide (NO), cyclooxygenase-2 (COX-2), prostaglandins E2 (PGE2) and other pro-inflammatory cytokines (iNOS) (Inkanuwat et al., 2019).

Nitric oxide (NO) inhibition has been used as indicators for the anti-inflammatory activity. The production of NO and its interaction with toxic agent are linked to several disease such as cancer, coronary heart disease, rheumatoid arthritis, asthma, and Alzheimer's. Suppressing the NO production can regulate the occurrence of inflammation (Inkanuwat et al., 2019). The lower of the NO production indicating the higher potential of the anti-inflammatory activity (Ke et al., 2021)

Pineapple is commonly associated with anti-inflammatory effect. This is due to is high content of bioactive compound such as bromelain and phenolic compound. Bromelain is complex mixture of several thiol endopeptidases and other related compounds obtained from the fruit, stem, and/or root of the pineapple plant (Varilla et al., 2021). Bromelain extract from pineapple rhizome shows anti-inflammatory activity by inhibit the production of inflammation mediators and cytokines (Insuan et al., 2021). The study on anti-inflammatory activity in 'Phulae' and 'Nanglae' pineapple peel extract has been conducted by Pongjanta and Chansiw (2019). The extracts are capable to inhibit the production of nitric oxide with varied strength, depending of the extract concentration. A study by Jusri et al. (2019) also found out that the core extract of pineapple has anti-hyaluronidase potentials. Despite the result is only comparing the extract with luteolin, it shows positive indication that pineapple could use to reduce the inflammation.

2.3 High Pressure Processing (HPP)

High pressure processing is one of a non-thermal technique in which the temperature is not the main factor that is used to eliminate both enzymes and microorganism (Barba et al., 2012). HPP mainly disrupt with the weaker, non-covalent bonds in enzymes, such as hydrogen bonds, but it does not affect the stronger covalent bonds, such as the peptide bonds that make up the enzyme's main structure. The higher enzyme inactivation is expected with higher temperature, pressure, and longer processing time during HPP (Silva & Sulaiman, 2022). The enzyme disruption also occurs on the microorganism in the product, in which disrupt the biochemical reaction which essential the survival and reproduction of the microorganism itself (Podolak et al., 2020). The lethal effect of HPP on the microorganism is not only due to the combination of changes on cell membrane permeability and the cell morphology, but also the interference of genetic mechanism on the microorganism's cell. The increased of cell permeability on the microorganism resulting in the outflow of the internal components of the cell (metal ions/internal solutes), injured the cell itself, and leads into the lack of nutrients for the cell, in which resulting in cell death (Sehrawat et al., 2020).

Source Bao Tou KeFA High Pressure Technology (2025)

Figure 2.4 High pressure processing machine

The development of high-pressure processing (HPP) technology was boosted by the consumer demand of high-quality convenience food. HPP machine (Figure 2.4) are proven to produce a microbial safe and stable product with improved quality characteristic both scientifically and commercially (Barba et al., 2015). HPP could enhance safety and extending shelf life with minimal influence on the sensory, physical, and nutritional properties of the food (Abera, 2019).

The novel feature of this technology, which could eliminate microorganism in room temperature or lower has become the one of the main attractiveness from this technology (Rastogi et al., 2007). As the result of this feature, the product could have better quality than thermal process product. Low temperature process will also preserve some bioactive compound that is heat-labile.

2.3.1 High Pressure Processing Principle

High pressure processing, or sometimes called pascalization is a non-thermal processing which using pressure for the treatment. The pressure used are between 100-700 MPa, depending the type of the food (Daher & Pérez-Lamela, 2017). The main mechanism of HPP is applying pressure to a product by using liquid as pressure transmission medium (Ke et al., 2021). The pressure itself are generated through mechanical pressure to the transmission medium (liquid, usually water) and consequently transmitted to the product. During the process, in the vessel contained the transmission medium and the product, the pressure will be held at constant rate at certain amount of time. This allowed the pressure to transmitted instantly and in uniform state throughout the food which will create more homogenous product. However, the increase of pressure applied will also slightly increase the processing temperature, 2-3^{o}C for every 100 MPa pressure increase (Daher & Pérez-Lamela, 2017).

2.3.2 High Pressure Processing in Pineapple

High pressure processing in pineapple product has been done commercially for quite a while. Most of the product are commercial pineapple juice. Table 2.4 show some study that applied HPP on the pineapple product and the result of the study.

Table 2.4 HPP treatment in pineapple product

Study by	Commodities	HPP treatment	Result
Chuensombat et al. (2019)	'Nanglae' Pineapple Juice	400 and 600 MPa, 5 mins	-maintain appearance, TSS, during cold storage
Wu et al. (2021)	Pineapple Juice	500 MPa, 10 mins	-retain original color, antioxidant activity, bioactive compounds, volatile compounds
Marcellini et al. (2007)	Pineapple puree	-	-improved the sensory quality compare with the commercial one
Alemán et al. (1996)	Pineapple juice	270 MPa, 40-400 secs	-inactivation of *Saccharomyces cerevisiae*
Kingsly et al. (2009)	Pineapple slice	50, 100, 300, 500 and 700 MPa at 25C for 10 min	- reduced the sample hardness, springiness, and chewiness - had no significant effect on cohesiveness of pineapple - alternative for hot water blanching, before dehydration

2.3.3 The Effect of High Pressure Processing on Food Quality

2.3.3.1 Microbial Reduction

In order to reduce the microbes on food, high pressure processing operation are divided into high pressure pasteurization (300-500 MPa, 1-5 mins, initial temperature 5-15^{o}C) for vegetative pathogen inactivation and high pressure sterilization (1-10 mins, initial temperature 70-90^{o}C, processing temperature 110-120^{o}C) for bacterial spores inactivation (Daher & Pérez-Lamela, 2017).

Studies in different fruit derivatives (juices, nectars, jams, purees, pastes, etc.) on the effect of HPP treatment revealed that the treatment is capable to destroy most microorganism (inactivation). However, it also depends on the application condition, type of microorganism, type of food, and the food structure itself. Yeast and

mold are less resistant to pressure compared to bacteria but most of them are inactivated by 400 MPa pressure (Daher & Pérez-Lamela, 2017).

2.3.3.2 Nutritional Improvement (Bioactive Compound)

There are a lot of study focusing on the beneficial effect of high pressure processing on the bioactive compound in various product. A review by Gamlath in 2011 found that HPP could retain some bioactive compound in fruit such as the phenolic compound, phenolic acid, and total flavonoid. Not only retaining some bioactive compound, in some fruit (blackberry, onion, and strawberry), the HPP increase the total phenolics compound (Gamlath, 2011). The result is similar with study on broccoli by Ke et al. (2021) which found that the total phenolic compound of the broccoli was increased after the HPP treatment.

The increase of phenolic compound after the high pressure treatment is due to the hydrophobic bonds disruptions in the cellular walls and cell membrane. The disruptions lead into higher rates of substance transfer and facilitating the solvent's penetration to the cell (Prasad et al., 2009). Studies involving measurement of HPP influence on bio accessibility of several compound found the different results as HPP depending on the magnitude of pressure, processing time and processing temperature present a great variability or bio accessibility of same and different compounds (Evrendilek, 2018).

2.3.3.3 Glycemic Index and Dietary Fiber

Several studies have been conducted to find the effect of high pressure processing on the food dietary fiber, especially the conversion of the insoluble dietary fiber to soluble dietary fiber. A study on okara (soybeans by-product) by Mateos-Aparicio et al. (2010), focused on the soluble fraction of fibre (SDF) found that the HPP treatment at 200 and 400 MPa, 30 and 60ºC could increase the amount of SDF more than 8 times, compare to the untreated product. The study also found that the increased pressure highly correlated with higher dietary fiber type conversion. Another study also found similar result. The HPP treatment at 600 MPa, 10 min, 22 and 55℃ on mango, orange, and prickly pear peels resulted in higher SDF fraction (Tejada-Ortigoza et al., 2017). However, both of the study only focuses on the dietary fiber fraction changes

after HPP treatment, which modify the IDF to SDF, without implying it with glycemic index (GI).

A study by Elizondo-Montemayor et al. in 2015 found that high-pressure processing treatment in mango puree resulted on lower GI and more favorable glycemic response. There are higher proportion of subject with low GI when they consumed the HPP-treated mango puree, compared to the non-treated one. It indicates that HPP processing has high potential in the dietetic management of insulin resistance, diabetes mellitus, and obesity. In the further studies by Elizondo-Montemayor et al. in 2020, they found that HPP treatment in commercial condition (592 MPa, 3 min) could reduce the GI by increasing the soluble dietary fiber (converting the insoluble dietary fiber to soluble dietary fiber) and the viscosity of the product which resulted in the slower and decreased glucose absorption rate, reduced glycemic response, and delayed gastric emptying activity during digestion.

2.3.3.4 Anti-Inflammatory Activity

Anti-inflammatory in some foods is usually related to the bioactive component in the food itself. Since the treatment could retain some bioactive compound in the food, then the anti-inflammatory activity might be higher as well. However, there is limited research on the effects of high-pressure processing (HPP) on anti-inflammatory activity in food, with most recent studies focusing on broccoli, mulberry fruit, and mushrooms.

The study by Ke et al. (2021) shows that the HPP treated broccoli at 400 MPa for 15 mins, which show higher isothiocyanates content, also show the higher inhibitory effect of nitric oxide (NO) production, indicating that HPP product has higher anti-inflammatory activity. Isothiocyanates are key bioactive compounds found in cruciferous vegetables. In broccoli, sulforaphane is the predominant isothiocyanate, followed by erucin. The compounds are heat-sensitives and HPP could preserve the compounds as well.

The study on mulberry fruit extract treated with high hydrostatic pressure (HHP) at 100 MPa for 4 hours at 50°C, which are high in bioactive compound such as anthocyanins and flavonols, demonstrated its anti-inflammatory effects by inhibiting various mediators and cytokines involved in the inflammatory process. This included

an inhibition in nitric oxide (NO) release in an in vitro model using lipopolysaccharide (LPS)-induced inflammation in RAW264.7 macrophage cells (Jung et al., 2019). Similar result also found on HPP assisted extract of the mycelia of *Grifola frondosa,* edible mushroom which widely consumed in East Asia. HPP treatment at 600 MPa for 5 mins shows stronger anti-inflammatory activities compared to the untreated mushroom extract (Wu et al., 2017).

2.4 Pineapple Puree as A Functional Food

Functional foods are food that has similar appearance as conventional food, to be consumed as part of usual diet, but has function beyond its basic nutrition. It may provide health effect and certain beneficial effect on one or more target function of the body that could reduce the risk of chronic disease (Gonçalves et al., 2022). There are five approaches to produce functional food such as eliminating the component that cause adverse effect when consumed, increasing the concentration of natural component which induce the predicted health effect on the body, adding beneficial component which not normally present on the food, replacing some component with other component that has beneficial effect, and increasing the bioavailability and stability of certain component which product functional effect (Henry, 2010).

Functional foods and beverages market are projected to reach USD 529.66 billion by 2028 (Fortune Business Insights, 2021). It targets a number of health objectives including enhancing weight loss, improving joint health, increasing muscle and bone strength, decreasing risk factors for cardiovascular disease and type 2 diabetes, enhancing digestion, and decreasing wrinkles (Spano, 2010). The current trend of the functional food and beverages are retaining the high level of the bioactive compound while maintaining the fresh like quality as much as possible (Gamlath, 2011).

There are several products using pineapple as the main material for functional food and beverages. One of the functional foods with pineapple juice based is the fermented *Lactobacillus* and *Bifidobacterium* strains (Nguyen et al., 2019). Another

example is the functional snack using Pineapple Puree, Sweet potato and Nata de Coco as the material (Rohaman, 2000).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Experimental Design

The overall experimental design was shown in Figure 3.1. The preliminary experiment involved the production of 'Phulae' pineapple puree using various treatment, including HPP at 400 and 600 MPa for 5, 10 and 15 mins, heat treatment, and the standard production method as control. These samples were then analyzed for quality attributes, bioactive compounds, and antioxidant activity analysis. Based on the initial results, selected treatments were further evaluated for changes in quality attributes, bioactive compounds, and antioxidant activity during storage, as well as for their effects on glycemic index and anti-inflammatory activity.

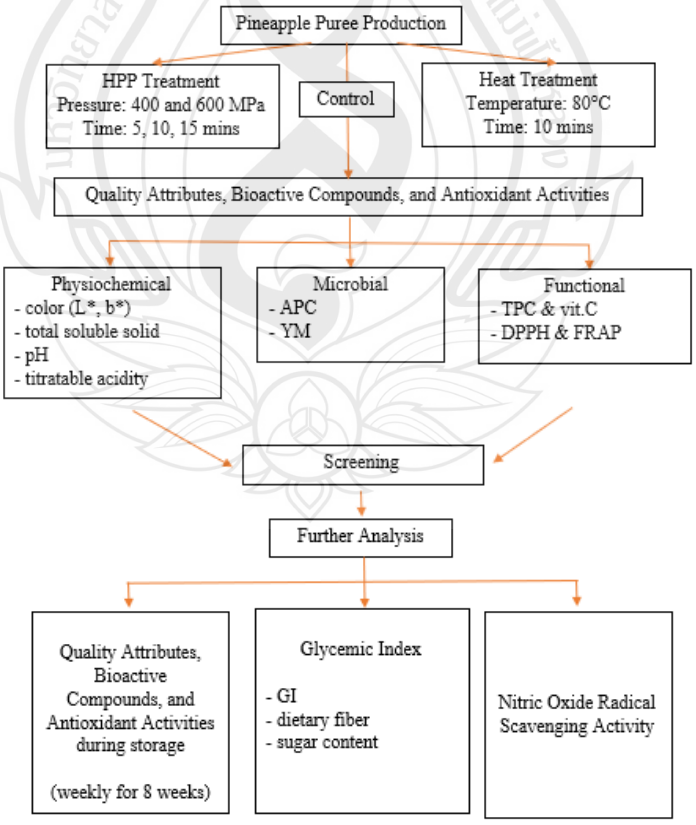


Figure 3.1 Overall experimental design

3.2 Materials

The raw material was fresh and fully matured (ripened) ‘Phulae’ pineapple (*Ananas comosus* L. Merr) with total soluble solid between 15-18 ^{o}Brix that cultivated in Chiang Rai area and processed in Mae Fah Luang University Laboratory. The ‘Phulae’ pineapple were purchased in the fresh packed peeled, whole form, and free from external defect.

3.3 Chemical

Indophenol dye, Folin Ciocalteu reagent, sodium hyaluronate, hyaluronidase, 1,1-diphenyl-1 picrylhydrazyl (DPPH), Trolox, 2,4,6-tripyridyl-S-triazine (TPTZ), N-(1-Naphthyl) ethylene-diamine dihydrochloride, hexadecyltrimethyl ammonium bromide (CTAB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Metaphosphoric acid was purchased from PanReac AppliChem (Castellar del Vallès, Barcelona, Spain). Acetic acid (CH_3COOH), sodium hydroxide (NaOH), hexane, hydrochloric acid (HCl), methanol, ethanol, and acetone were purchased from Qrec (Muang, Chonburi, Thailand). Peptone and sodium nitroprusside (SNP) were purchased from Himedia (Thane, Maharashtra, India). Ferrous sulfate ($FeSO_4$) was purchased from Ajax Finchem (Seven Hills, NSW, Australia). Pepsin, pancreatin, and amyloglucosidase enzyme were purchased from Megazyme (Bray, Co. Wicklow, Ireland). All the chemical and reagents used in the experiments were analytical grade.

3.4 Methods

3.4.1 ‘Phulae’ Pineapple Puree Production and Storage

The pineapple puree processing started with the washing process using distilled water to remove external dirt or soil particles. All the sample batch were screened for any bruises, and randomly checked for the total soluble solid around 15-18 °Brix to make sure the similar maturity among the ‘Phulae’ pineapple that were used as raw

material. The production was followed Chakraborty et al. (2014) with modification. The whole pineapples were cut into medium pieces using stainless-steel knife and blended (speed at level 5 for 30 s) by commercial blender (Philips Blender, model HR2120, Indonesia). All the ground sample batch of pineapples including control (without any treatment), and samples to be treated with high pressure processing (HPP) were packed in LLDPE pouch (8 × 14 cm) (65 g /pouch). However, a portion of ground pineapple sample was kept in a sterile stainless-steel container covered with lid for thermal treatment and post heat processed samples were also packed in LLDPE pouch.

Processed samples in pouch (fresh and treated) were stored at 5±1°C dark room before analysis.

3.4.2 High Pressure Processing and Thermal Treatment of 'Phulae' Pineapple Puree

These processes were based on the modification of Elizondo-Montemayor et al. (2020) application of HPP in mango puree and using thermally processed sample (2 mins at 70°C) as control. For these studies, packed pineapple puree samples were subjected to high-pressure processing at 400 MPa and 600 MPa for 5, 10, and 15 min using a high-pressure unit (Bao Tou KeFa High Pressure Technology Co., Ltd., China). The processing temperature was maintained at 25°C, with distilled water as the transmission medium. For the heat treatment sample, fresh pineapple puree was also treated at 80°C for 10 min in a commercial pot and cooled before packaging. All samples were stored at 5±1°C prior to analysis and extraction of supernatants. For the extraction, each sample was mixed with distilled water at a 1:5 ratio (g/v) using a vortex mixer (model Genie 2, Scientific Industries, Inc., USA) for 1 min. The mixture was then centrifuged at 15,000 × g for 30 min at 4°C (Sorvall Legend X1R, Thermo Fisher, Germany), and the supernatant was collected for further analysis.

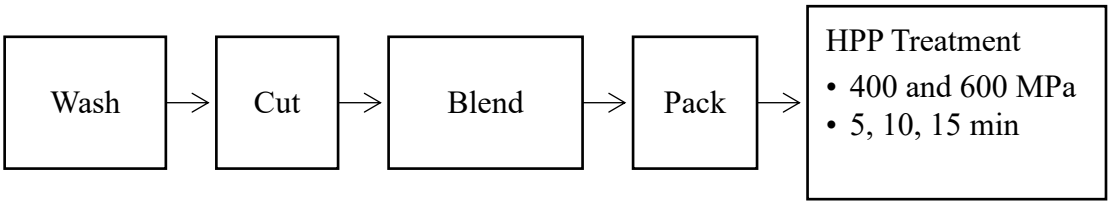


Figure 3.2 Pineapple puree production and HPP processing step

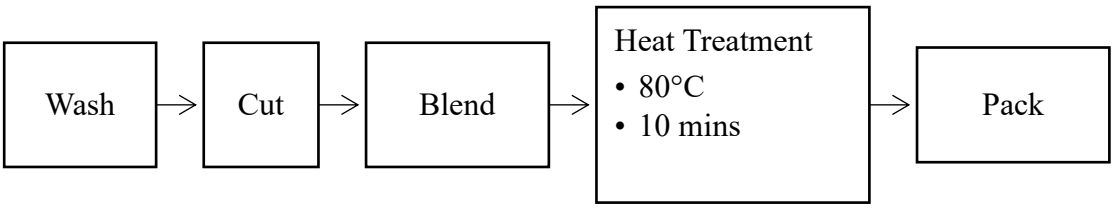


Figure 3.3 Pineapple puree production and HT processing step

3.4.3 Effect of HPP on Quality Attributes, Bioactive Compounds, and Antioxidant Activity of 'Phulae' Pineapple Puree

3.4.3.1 Physiochemical Analysis

1. Color

CIE L*a*b* color value were measued in triplicate using color spectrophotometer CR-10 (model CM-600d, Konica Minolta, Inc., Japan). However, only L* (lightness) and b* (yellow-blue) were used to observe of the pineapple puree's main color. The a* (green red) will be used in the shelf life analysis (storage) to calculate the total color change. The total color change (ΔE*) at *n*th week during observation will be calculated using equation (1) according to Chakraborty et al. (2016).

$$\Delta E^* = \sqrt{(L_1^* - L_0^*)^2 + (a_1^* - a_0^*)^2 + (b_1^* - b_0^*)^2} \quad (1)$$

2. Total Soluble Solid

Processed pineapple puree was directly analyze using digital hand refractometer (PAL-1, ATAGO, Japan) to determine the soluble solid content which will be expressed in ^{o}Brix.

3. pH

Processed pineapple puree was directly analyze using the pH meter (FiveEasy™ FE20-1, Mettler-Toledo AG, Switzerland). Prior to the analysis, the pH meter will be calibrated using pH 4.0, 7.0, and 12.0 standard buffer solution.

4. Titratable Acidity (TA)

Titratable acidity (TA) was determined by directly analyzed the sample using acidity meter (PAL-Easy ACID F5, ATAGO, Japan). TA results were expressed as % titratable acidity (TA).

3.4.3.2 Microbial Analysis

1. Total Aerobic Count (3M Petrifilm^{TM} Aerobic Count Plate)

The aerobic microbial count was determined by using 3M PetrifilmTM Aerobic Count Plate (AOAC official method 990.12, 2002). The sample were diluted 1:10 in 0.1% peptone water. The dilution (1 mL) then transfered into the petrifilm which is placed in the flat surface, then spread using spreader device. The petrifilm left undistributed for 1 min to let the gel set before incubated in stacks at 35 ± 1ºC for 48 ± 3 hours. The total colonies were count and calculated to obtain the total aerobic count in log CFU/ml.

2. Yeast and Mold (3M Petrifilm^{TM} Yeast and Mold Count Plate)

The yeast and mold count were determined by using 3M Petrifilm^{TM} Yeast and Mold Count Plate (AOAC official method 997.02, 2002). The sample were diluted 1:10 in 0.1% peptone water. The dilution (1 mL) then transfered into the petrifilm which is placed in the flat surface, then spread using spreader device. The petrifilm left undistributed for 1 min to let the gel set before incubated in stacks at 20-25ºC for 5 days. The total colonies were count and calculated to obtain the total aerobic count in log CFU/ml.

3.4.3.3 Functional Properties Analysis

1. Bioactive Compounds: Total Phenolic Compound

The total phenolic compound was determined by colorimetric assay using Folin-Ciocalteu phenol reagent (International Organization for Standardization (ISO) 14502-1, 2005) with some modification. The analysis was following the diagram below (Figure 3.4) and calculated using equation (2):

$$Total\ Phenolic\ Compound\ (mg\ GAE/100ml) = C \times \mathrm{V} \times \mathrm{DF} \quad (2)$$

Where C is concentration of gallic acid from calibration curve (mg/ml), V is the sample volume (ml), and DF is the dilution factor.

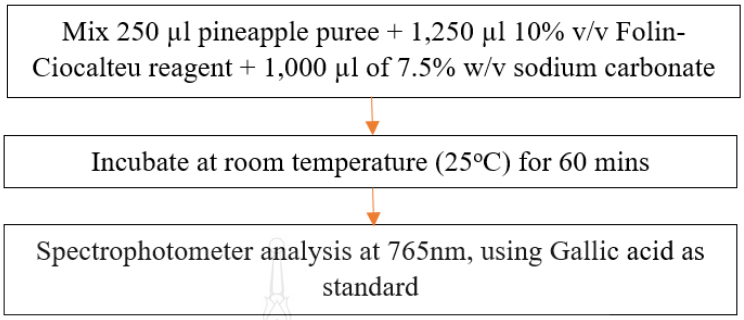


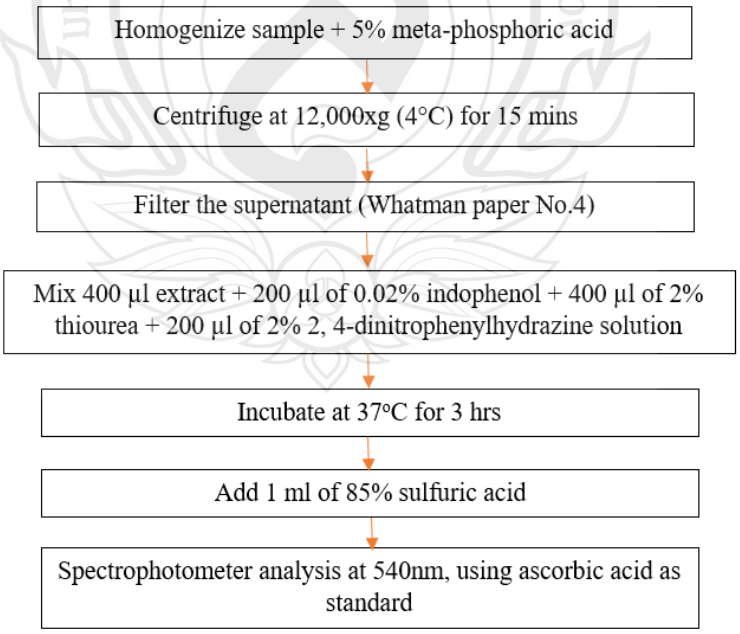
Figure 3.4 Total phenolic compound analysis (ISO 14502-1, 2005)

2. Bioactive Compounds: Vitamin C (Ascorbic Acid Content)

The vitamin C (ascorbic acid) was determined following Roe et al. (1948) with some modification. The analysis was following the diagram below (Figure 3.5) and calculated using equation (3):

$$Vitamin\ C\ (mg\ per\ 100gr) = \frac{C \times V}{W} \times \text{DF} \times 100 \qquad (3)$$

Where C is the concentration of ascorbic acid from the calibration curve (mg/ml), V is the extract volume (ml), W is weight of the sample (g) and DF is the dilution factor.



Source (Roe et al., 1948)

Figure 3.5 Ascorbic acid analysis

3. Antioxidant Activity: DPPH radical scavenging activity assay

The antioxidant capacity of the pineapple puree was determined using 1,1-diphenyl-1 picrylhydrazyl (DPPH) free-radical scavenging assay described by Khalaf et al. (2008) with some modification. A 50 µl of pineapple puree were mixed with 1,950 µl 60µM DPPH solution (dissolved in 95% methanol) before incubated in dark condition for 30 mins. Spectrophotometer were used to measure the mixture's absorbance at 517nm and using Trolox Equivalent as the standard and methanol as the blank. The DPPH radical scavenging activity were reported as µmol TE (trolox equivalent) per 100g of the pineapple puree. The calculation were following equation (4):

$$DPPH\ \ (\mu mol\ TAE/100\ g) = C\ \times\ \mathrm{V} \times\ \mathrm{DF} \qquad (4)$$

Where C is Trolox concentration from calibration curve (µmol /ml), V is the sample volume (ml), and DF is the dilution factor.

4. Antioxidant Activity: Ferric reducing antioxidant power (FRAP) capacity assay

The antioxidant activity of pineapple puree was determined using ferric reducing antioxidant power (FRAP) capacity assay as described by Benzie & Szeto (1999). The FRAP reagent was prepared fresh by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) in 40 mM Hydrochloric Acid (HCl) and 20 mM Iron (III) chloride (FeCl3) in ratio of 10:1:1 (v/v/v). The pineapple puree (400 µl) was mixed with the 2.6 ml FRAP reagent and incubated at 37ºC for 30 min. Spectrophotometer were used to measure the mixture's absorbance at 595 nm and using ferrous sulfate equivalent ($FeSO_4$) as the standard and water as the blank. The DPPH radical scavenging activity were reported as µmol ferrous sulfate equivalent ($FeSO_4$) per 100ml of the pineapple puree. The calculation were following equation (5):

$$FRAP\ \ (\mu mol\ FeSO_4/100\ ml) = C\ \times\ \mathrm{V} \times\ \mathrm{DF} \qquad (5)$$

Where C is ferrous sulfate equivalent ($FeSO_4$) concentration from calibration curve (µmol /ml), V is the sample volume (ml), and DF is the dilution factor.

3.4.4 Effect of HPP on 'Phulae' Pineapple Puree's Quality Attributes, Bioactive Compounds, and Antioxidant Activity During Storage

The pineapple puree was produced following the method on section 3.4.1 and 3.4.2. Samples were produced in big batch and stored at 5±1°C in a dark condition. Sample analysis was done weekly for 8 weeks period, started from the beginning of the experiment (week 0 to week 8). The analysis including physiochemical, microbial, and functional properties analysis was following the method mentioned on section 3.4.3.

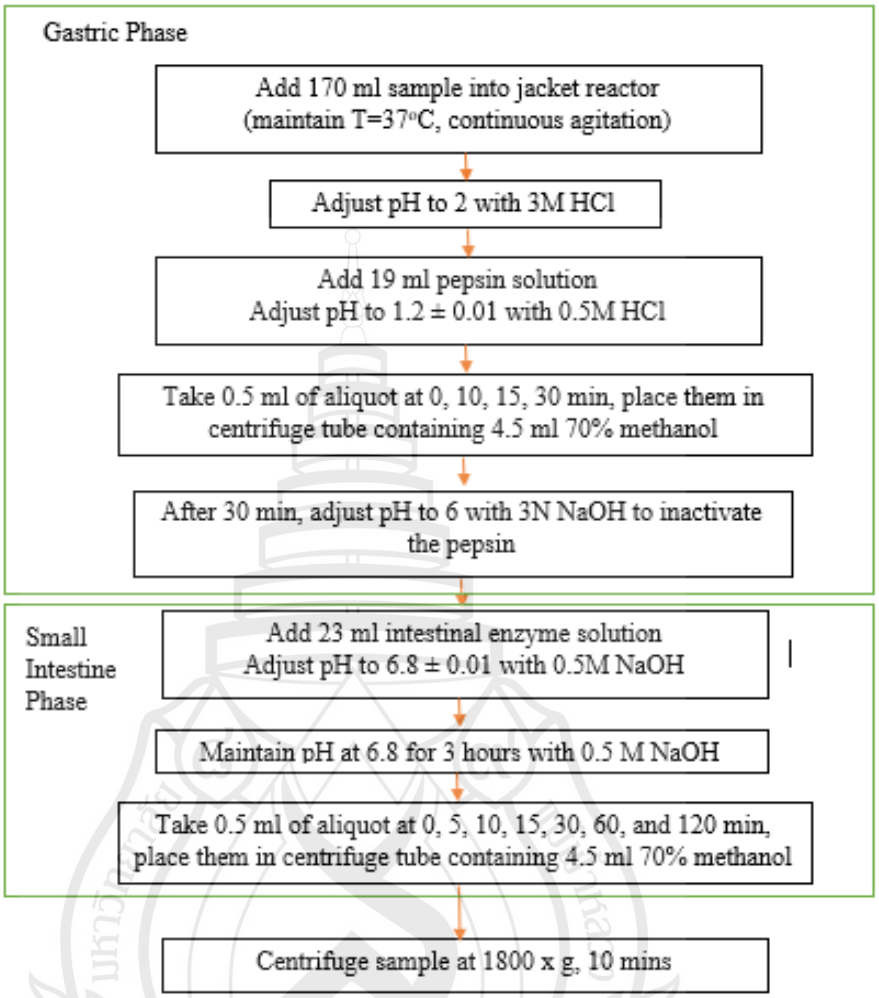
3.4.5 Effect of HPP on 'Phulae' Pineapple Puree's Glycemic Index

Determination of the glycemic index in the pineapple puree were using the *in vitro* digestion model. The dietary fiber determination was also conducted to support the overall data analysis.

3.4.5.1 Glycemic Index

The glycemic index analysis was using the two-stage *in vitro* digestion model (Tamura et al., 2016) with some modification. The *in vitro* digestion was imitating the digestion process in gastric and small intestine phase in the body. Sample was added some enzyme that is originally playing part in human digestion system, such as pepsin, pancreatin, and amyloglucosidase. The digested sample then analyzed for the glucose content. The method described in the figure 3.6.

The glucose content was examined using D-glucose assay kit (GOPOD Format K-GLUK 07/11, Megazyme International). The supernatant (0.1ml) were mixed with invertase/amyloglucosidase solution (pH 5.20) in a test tube then incubated at 37°C for 10 min in water bath. The mixture (0.1ml) was mixed with GOPOD solution (3ml), followed by another incubation at 50°C for 20 min in water bath. The absorbance of the mixture was measured using spectrophotometer at 510 nm.



Source Tamura et al. (2016)

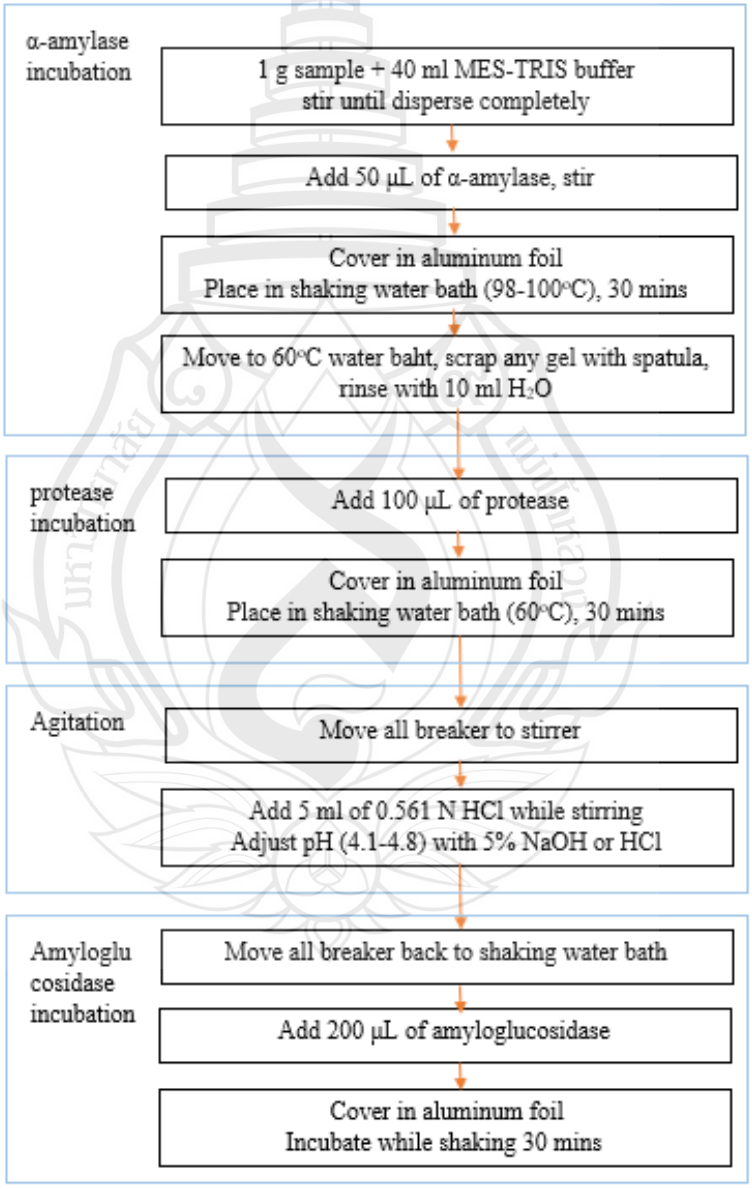
Figure 3.6 *In vitro* analysis

According to Leoro et al. (2010), the glucose digestion rate expressed through the percentage of glucose in each sample at specific interval. hydrolysis curve was built and the area below the hydrolysis curve was calculated (AHC). The hydrolysis index (HI) for each sample then calculated as ratio between AHC of each sample and AHC of white bread. The estimated GI value was calculated with the equation (6) introduced by Goni et al. (1997) as mentioned below

$$Estimated\ GI = 39.71 + 0.549\ HI \quad (6)$$

3.4.5.2 Dietary Fiber

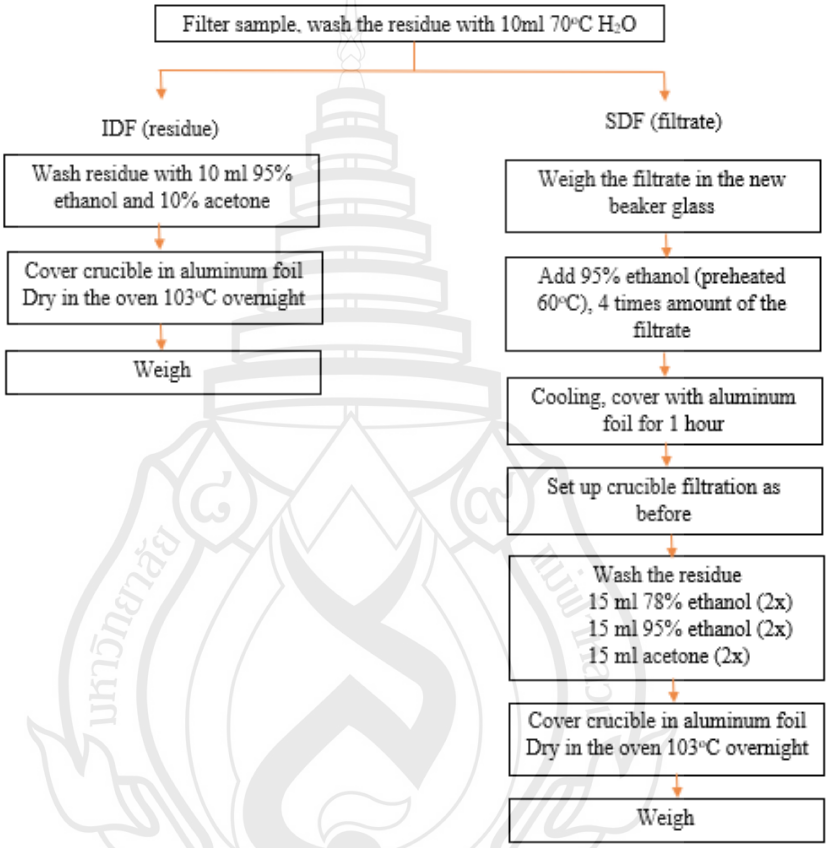
The total dietary fiber (TDF) determination was based on AOAC 991.43 and AOAC 985.29 method (Elizondo-Montemayor et al., 2020) using total dietary fiber assay kit from Megazyme. The TDF was accumulation of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) which were obtained during the analysis. The analysis was divided into 2 step, enzyme analysis and the filtration which is described by the figure 3.7 and 3.8.



Source (Elizondo-Montemayor et al., 2020)

Figure 3.7 Dietary fiber analysis (1)

The sample mixture went to the filtration process to separate the IDF and SDF. The filtration was using the crucible which covered in 1 g celite. Prior to the use, the crucible and celite needed to be dried at 103 ℃ for an hour then redistribute the celite by 15 ml of 78% EtOH with the help of vacuum suction. The further step is described in the figure 3.8.



Source (Elizondo-Montemayor et al., 2020)

Figure 3.8 Dietary fiber analysis (2) (Elizondo-Montemayor et al., 2020)

3.4.5.3 Sugar Profile

The sugar profile analysis was analyzed at an external laboratory (Central Lab (Thailand) Co., Ltd.) using their in-house method, based on the Compendium of Method for Food Analysis (2003).

3.4.6 Effect of HPP on 'Phulae' Pineapple Puree's Nitric Oxide Radical Scavenging Activity

The nitric oxide radical scavenging activity will be analyzed using method by Hazra et al. (2008) with modification. The nitric ions will be generated from the interaction between oxygen and aqueous sodium nitroprusside (SNP), quantified by Griess Illosvoy reaction. The detailed steps will be described in figure 3.9.

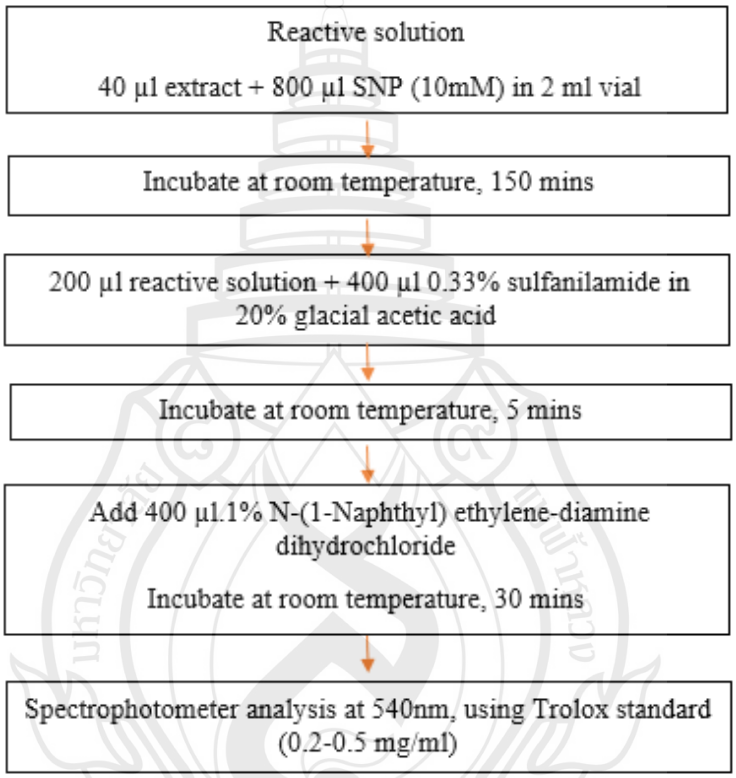


Figure 3.9 Nitric oxide radical scavenging activity analysis

3.4.7 Statistical Analysis

All the data gathered were analyzed using analysis of variance (ANOVA) and Duncan's multiple range test and at 95% ($p<0.05$) confident level. IBM SPSS Statistic 26 will be use as the software to analyze the data and all data were presented as means ± standard deviation (SD). For the analysis, all test were conducted in triplicate.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Effect of HPP on Quality Attributes, Bioactive Compounds, and Antioxidant Activity of 'Phulae' Pineapple Puree

4.1.1 Quality Attributes: Physiochemical

The physiochemical properties of the 'Phulae' pineapple puree that were observed are the color, pH, total soluble solid, and titratable acidity. As shown in Figure 4.1, all the samples (both fresh and processed) exhibit a homogenous appearance with varying shades of yellow color. Although an inexperienced observer might perceive all samples color as nearly identical, a more detailed color analysis would likely provide better insights.

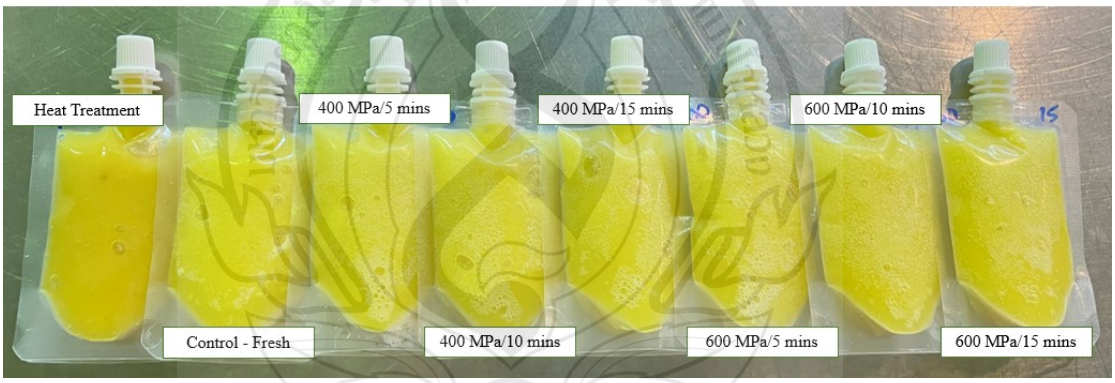


Figure 4.1 'Phulae' pineapple puree in clear packaging

'Phulae' pineapple puree's color were observed through its L*and b* values from CIELAB system. L* represents the lightness (0 to 100) with 0 being black and 100 being white while b* is represent yellow when it has positive (+) value and blue when it has negative (-) value (Nielsen, 2017). The 'Phulae' pineapple puree has L* value ranging from 36.62 to 46.09 and b* value ranging from 20.31-30.34 (figure 4.2). The L* of the fresh sample is the highest (46.09) while HT sample has the lowest value (36.62) which mean that it has darker color compared to fresh and HPP treated sample.

Despite the significant different ($p<0.05$) between fresh sample and HPP treated sample, there is no significant different in term of lightness among the HPP treated sample with L* value around 40. The darker color of HT sample were induced by non-enzymatic browning, such as Maillard reaction (Niu et al., 2022).

The b* value (yellowness) of the fresh and HT sample were similar, but significantly lower ($p<0.05$) in HPP treated sample, which means that HPP could slightly decrease the yellowness of the puree. The higher pressure (HPP 600) shows lower b* value (20.31-23.39) compared to the lower pressure (HPP 400, 22.27-25.40) and the longer the treatment also resulted in lower b* value. The decreasing of b* value were happened in both HPP 400 and HPP 600 samples in which the 15 mins treatment has the lowest b* for each pressure. This is due to pigment degradation, particularly carotenoids which has wide range of color from yellow, orange, to red (Chandra et al., 2021). The result contradict with the previous studies in which HPP at optimum condition could retains the total carotenoids in the food product such as pineapple juice and passion fruit juice (Wu et al., 2021; Niu et al., 2022). In passion fruit juice, HPP could induce the enzymatic reaction of β-ring hydroxylase, resulting conversion of β-cryptoxanthin (R-isomer to β-carotene) to zeaxanthin (Niu et al., 2022).

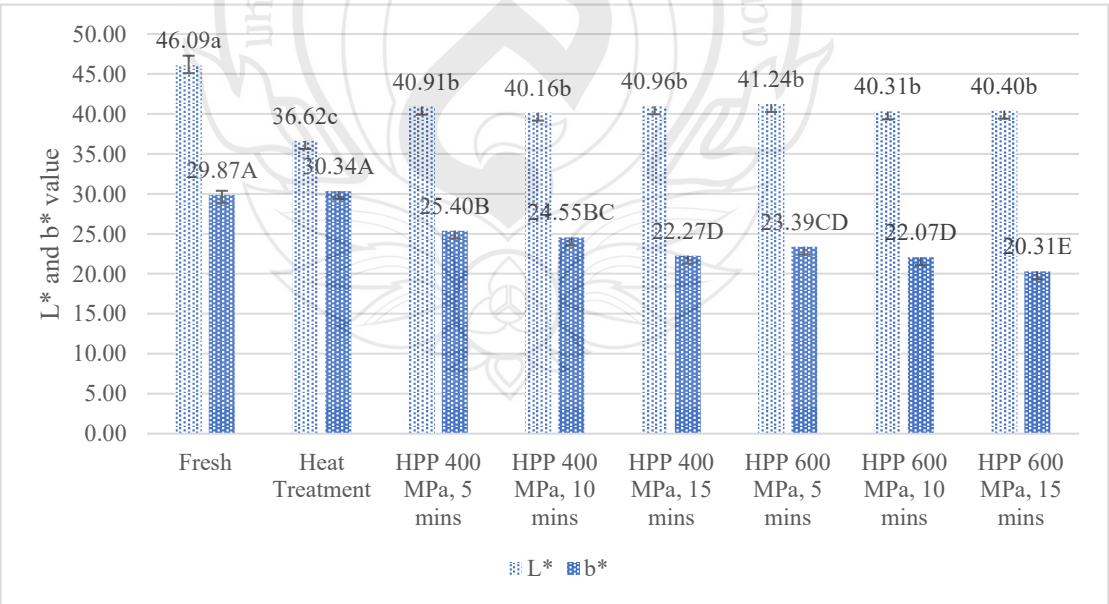


Figure 4.2 Color analysis of 'Phulae' pineapple puree

Color and texture are important part of principal quality attributes, besides flavor, which have huge roles in acceptance of food product (Nielsen, 2017). Based on the physiochemical analysis, compared with HT sample, the HPP treated sample has more similar characteristic to the fresh sample, which would indicate that the product itself has gone to minimal processed and would be more preferred by consumer. Despite the lack of sensory analysis in this studies, previous studies on the effect of HPP on juices and smoothies in sensory attributes shows that HPP could preserved the sensory properties better than HT and enables the 'fresh-like' quality product (Song et al., 2022)

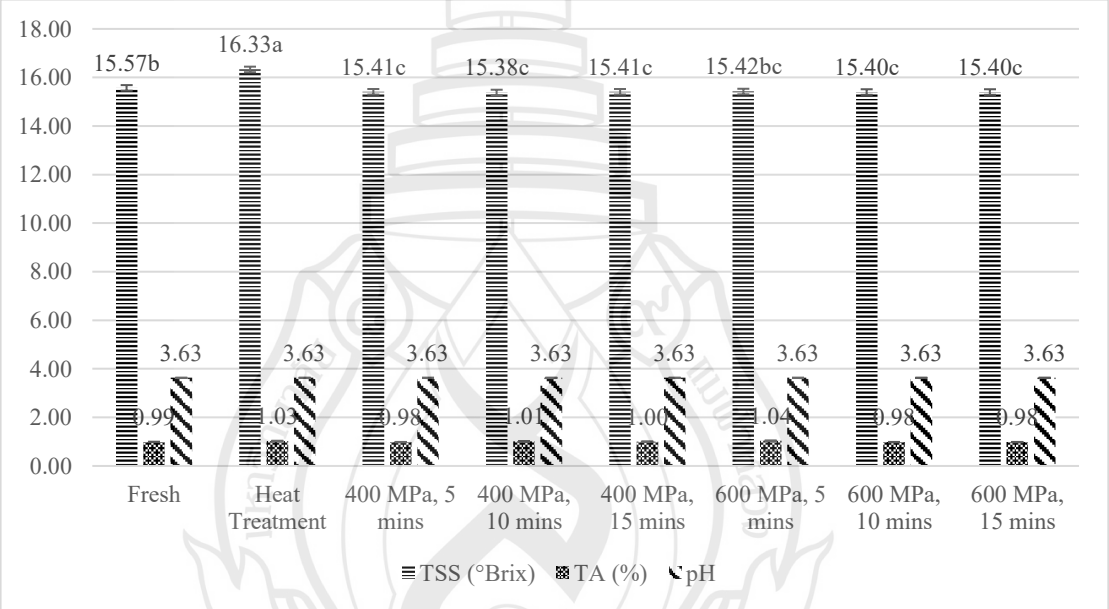


Figure 4.3 Physiochemical properties of 'Phulae' pineapple puree

There is no significant difference ($p<0.05$) in term of pH and titratable acidity among fresh, HT and HPP treated samples. The pH are around 3.63 with titratable acidity ranging from 0.98 to 1.04%. Adisak and Jintana (2011) reported similar value with Smooth Ceyenne pineapple pH around 3.4-4.5. Previous studies in pineapple juice also confirmed that there is no significant different in term of pH and titratable acidity after HT and HPP treatment with pH around 4.16-4.20, and titratable acidity ranged around 0.92-0.95% (Wu et al., 2021). Besides pineapple juice, the same impact of HT and HPP also found in orange juices (Ambreen et al., 2023).

There is significant difference in total soluble solids (TSS) between samples. The HT sample has the highest TSS value (16.33), followed by fresh sample (15.57)

and HPP treated sample (15.38-15.41). The higher TSS value in HT sample is due to the water evaporation during the heating process. Similar report in HPP treated mango puree found that there is a slight decrease in TSS from 15.83 to 15.70 (Elizondo-Montemayor et al., 2020). In contrast, Wu et al. (2021) found that both HT and HPP had no significant effect on TSS. However, their heat treatment lasted only 3 mins, which is much shorter than the 10-minute duration used in our studies.

4.1.2 Quality Attributes: Microbial

The initial aerobic plate count (APC) and yeast and mold (YM) for fresh 'Phulae' pineapple could be seen in Table 4.1, were 4.82±0.03 and 4.12±0.04 log CFU/mL, respectively. This indicates that fresh sample have high microbial safety risk. Following the HT and HPP, the microbial count decreased to less than 2.48±0.00 (APC) and 2.18±0.00 (YM) log CFU/mL, reducing the microbes by almost 2 log unit. This results consistent with the previous study in pineapple juice which HPP at 500 MPa for 10 mins and HT at 95°C for 3 mins could reduce more than 4 log unit of microbes (Wu et al., 2021) and passion fruit puree which HPP at 600 MPa for 5 mins and pasteurization (PT) at 85°C for 30 secs could reduce more than 3 log unit of microbes (Niu et al., 2022).

The lethal effect of the HPP on the APC and YM could be due to simultaneous effect of cell membrane changes, the cell morphology alteration, biochemical disruptions, and the interference in genetic mechanism which occur in cell microorganism. The microorganism cell membrane is fluidic and consist of proteins and phospholipid bilayer, separating the interior cell from external environment. HPP could increase the permeability of this membrane (due to the breakdown of the non-covalent bonds), damaged the cell membrane, resulted in the leaking of some internal solution, such as the adenosine triphosphate (ATP) which is essential substance for the cell. The lack of ATP lead into the inability of the microorganism for protein synthesize which eventually affect the metabolic process of microorganism (Sehrawat et al., 2020).

Table 4.1 Microbial analysis for 'Phulae' pineapple puree

Treatment	Aerobic Plate Count log CFU/ml	Yeast and Mold log CFU/ml
Fresh	4.82 ± 0.03^{a}	4.12 ± 0.04^{A}
Heat Treatment	$<2.48\pm0.00^{b}$	$<2.18\pm0.00^{B}$
HPP 400 MPa, 5 mins	$<2.48\pm0.00^{b}$	$<2.18\pm0.00^{B}$
HPP 400 MPa, 10 mins	$<2.48\pm0.00^{b}$	$<2.18\pm0.00^{B}$
HPP 400 MPa, 15 mins	$<2.48\pm0.00^{b}$	$<2.18\pm0.00^{B}$
HPP 600 MPa, 5 mins	$<2.48\pm0.00^{b}$	$<2.18\pm0.00^{B}$
HPP 600 MPa, 10 mins	$<2.48\pm0.00^{b}$	$<2.18\pm0.00^{B}$
HPP 600 MPa, 15 mins	$<2.48\pm0.00^{b}$	$<2.18\pm0.00^{B}$

Note Value expressed mean ± standard deviation. Data of different alphabets in the same column were different with statistical significance ($p<0.05$)

4.1.3 Bioactive Compounds: Total Phenolic Compound and Vitamin C

The total phenolic compounds (TPC) and total vitamin C (ascorbic acid) of the fresh and treated sample are shown in Table 4.2. The highest level of TPC were noted in pineapple puree that subjected to 600 MPa for 10 mins which is 304.77±1.56 mg GAE/100g DW, 7.5% higher than the fresh sample (283.60±2.05 mg GAE/100g DW) while the heat-treated sample showed a significant ($p<0.05$) decrease of 8.9% (258.24±1.56 mg GAE/100g DW). HPP treatment at 400 MPa showed significant ($p<0.05$) higher level of TPC (4.3-5.6%) compared with fresh sample, whereas HPP treatment at 600 MPa for 5 and 15 mins has no significant effect on the pineapple puree. There is no significant difference in term of vitamin C between the fresh sample and sample which subjected to 400 and 600 MPa for 5, 10 and 15 mins with vitamin C value around 19.51-21.29±0.16-2.02 mg/100g DW. In the other hand, heat-treated sample showed significant ($p<0.05$) lower value at 15.09±0.48mg/100g DW which attributed

to 22.7% decrease from the fresh sample. The lower value of TPC and vitamin C on the heated sample is more likely due to the degradation of phenolic compounds and ascorbic acid due to the high temperature. Kumar et al. (2023) reported the decrease of TPC and vitamin C in citrus juices after different range of heat treatment (70-100°C for 15.30 and 45 mins). The increase of TPC in certain HPP treated sample might be due to the plant cell matrix or cell wall disruption, induce by HPP which enhance the extraction of the bioactive compounds (Ketnawa et al., 2021). Higher extraction of bioactive compound were also reported in non-astringent persimmon fruit (Ketnawa et al., 2021). Besides, other studies reported that HPP also did not altered total phenolic and vitamin C concentration in mango puree (Elizondo-Montemayor et al., 2020).

Table 4.2 Bioactive compounds in 'Phulae' pineapple puree, treated with thermal and high pressure processing.

Sample	Fresh	Heat Treatment	HPP					
			400 MPa			600 MPa		
			5 mins	10 mins	15 mins	5 mins	10 mins	15 mins
Total Phenolic Compounds	283.60	258.24	295.88	298.88	299.39	294.11	304.77	285.71
(mg GAE/100g DW)	±2.05^{ab}	±1.56^{b}	±3.70^{a}	±4.16^{a}	±3.07^{a}	±1.65^{ab}	±1.56^{a}	±3.76^{ab}
Vitamin C	19.51	15.09	19.45	19.67	20.70	19.35	21.29	19.55
(mg/100g DW)	±1.78^{a}	±0.48^{b}	±1.91^{a}	±2.02^{a}	±0.16^{a}	±1.90^{a}	±0.87^{a}	±1.97^{a}

***Note** Value expressed mean ± standard deviation. Data of different alphabets in the same row were different with statistical significance ($p<0.05$)

4.1.4 Antioxidant Activity: DPPH and FRAP

The effect of HPP and heat treatment on 'Phulae' pineapple puree antioxidant activity was determined by two different assay (DPPH and FRAP) which have different oxidant and reaction mechanism to obtain the complete antioxidant profile (González-Peña et al., 2013). As shown in figures 4.4 and 4.5, HPP treatment has the same trends in which the higher pressure and longer period of HPP resulted in higher value not only in DPPH but also FRAP. DPPH value in pineapple puree which subjected to 600 MPa for 15 mins (1271.79±67.50 mmol Trolox/100g DW) is 14.7% higher than the fresh sample (1108.88±8.10 mmol Trolox/100g DW) while HT sample has the lowest value (1056.28±28.19 mmol Trolox/100g DW) but no significant different with the fresh sample. FRAP value in pineapple puree which subjected to 600 MPa for 15 mins also has significantly ($p<0.05$) highest value (2255.93±88.86µmol $FeSO_4$/100g DW), which is 12.7% higher than the fresh sample (2001.93±5.41µmol $FeSO_4$/100g DW) while heat-treated sample has significantly ($p<0.05$) lowest value (1730.75±69.35µmol $FeSO_4$/100g DW), which is 13.5 % lower than the fresh sample. HPP at 400 MPa for 5 and 10 mins has no significant effect on the FRAP value.

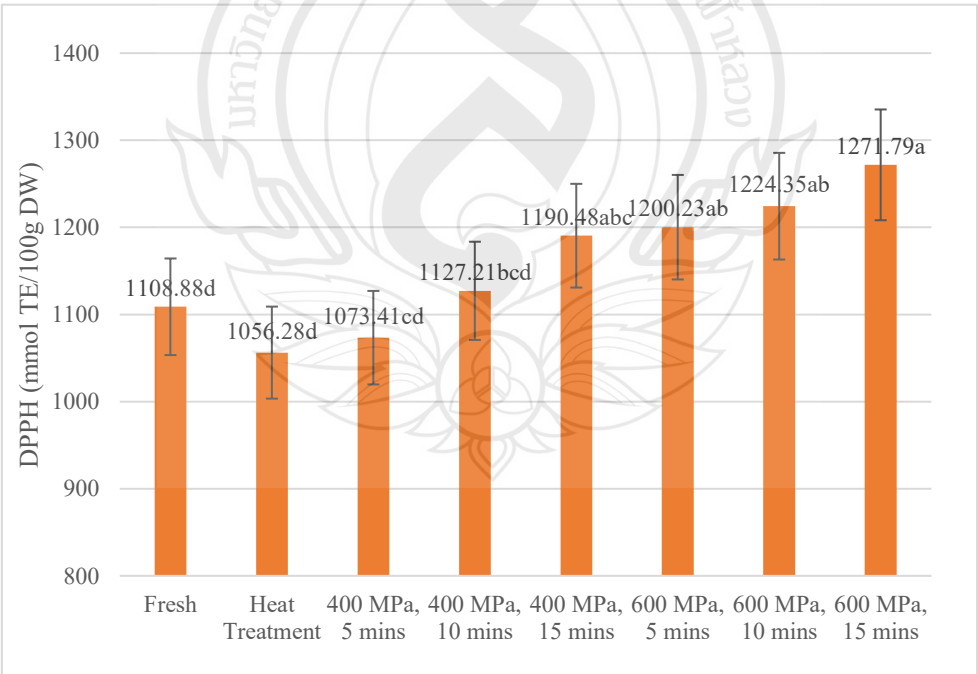


Figure 4.4 DPPH radical scavenging activity of 'Phulae' pineapple puree

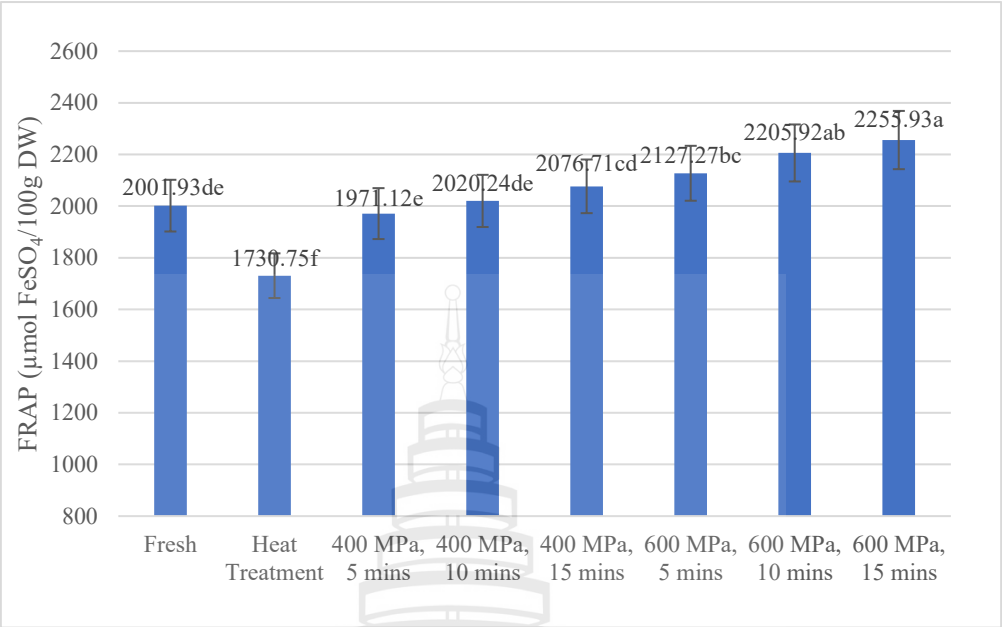


Figure 4.5 FRAP antioxidant capacity of 'Phulae' pineapple puree

The increase of antioxidant activity is typically due to the presence of the bioactive compound in the sample. There is a high significant correlation between antioxidant activity (ABTS, DPPH, FRAP, TBARS) and phenolic compounds (total phenolic, total flavonoids, and total flavanols) (Li et al., 2011). This also indicates that samples with lower levels of bioactive compounds, such as those subjected to heat treatment, will have lower antioxidant activity. In these studies, even though bioactive compounds and vitamin C were retained (and even higher at some sample), antioxidant activity increased with longer duration and higher pressure treatments. This suggests that other bioactive compounds might be contributing to the antioxidant activity in pineapple puree. Domínguez et al. (2018) noted that pineapple also contains β-carotene and flavonoids, which were not included in these studies.

4.2 Screening process for 'Phulae' Pineapple Puree

For the screening purposed, color-coded table was created as seen in Table 4.3 based on the result in section 4.1. Darker color shows the higher number while lighter color shows the lower number in the same row. Similar color in the same row shows no significant different observed in term or result and can be assumed as the same result.

Table 4.3 Color-coded table for screening of the optimum HPP treatment of 'Phulae' pineapple puree

Parameter		Fresh	HT	HPP 400 MPa, 5 mins	HPP 400 MPa, 10 mins	HPP 400 MPa, 15 mins	HPP 600 MPa, 5 mins	HPP 600 MPa, 10 mins	HPP 600 MPa, 15 mins
Physiochemical	L*								
	b*								
	TSS								
	pH								
	TA								
Microbial	APC								
	YM								
Antioxidant Capacity	DPPH								
	FRAP								
Bioactive Compounds	vit C								
	TPC								

Note Color intensity in the same row expressed different value with darker color showed higher value and lighter color showed lower value. Different color represents statistical significance difference ($p<0.05$) using Duncan's multiple range test.

Fresh and heat-treated sample (HT) were still chosen as control and the main comparison with the commercial product. However, for the further analysis, only 1 treatment from each 400 and 600 MPa of HPP treated sample that was chosen. Based on Table 4.3, in term of physiochemical and microbial, there is no difference in between all the HPP treated sample, expect for b* in term of yellowness that became smaller with higher pressure and longer time of HPP. For antioxidant capacity, the higher

pressure and longer time resulted in higher number of DPPH and FRAP while for bioactive compound, the number of vit. C and TPC are stable. Aiming for higher result, since the result for the antioxidant capacity and bioactive compound for 10 and 15 mins are similar, the 10 mins treatment for both pressures were chosen. The shorter time will save more time and energy used during the treatment.

4.3 Effect of HPP on 'Phulae' Pineapple Puree's Quality Attribute, Bioactive Compounds and Antioxidant Activity During Storage

As a continuous part from Chapter 4.1, similar properties are observed throughout 8 weeks period for fresh sample, heat treated sample (HT), and high pressure sample (HPP 400 and 600 MPa for 10 mins).

4.3.1 Quality Attributes: Physiochemical

The physiochemical properties of the 'Phulae' pineapple puree that were observed are the color, pH, total soluble solid, and titratable acidity.

4.3.1.1 Color

Color analysis result for the 'Phulae' pineapple puree are presented in figure 4.6. The L* value of fresh and HT sample is stable during the storage. However, the HPP treated sample L* value is significantly lower ($p<0.05$) overtime during the storage. The b* value for all sample also significantly reduce ($p<0.05$) during the storage. One week storage has the lowest ($p<0.05$) color change (ΔE*) in all treatment, ranging around (1.41-12.00), with HT sample has the lowest (ΔE*) followed by fresh sample and HPP samples. The value increase in second week but remain stable until the end of the observation (week 8). During the observation HT samples show the smaller color changes compared with the other samples. This is probably due to the complete inactivation of the enzyme which could resulted in better preserving of the color itself. On the other hand, the color changes for HPP treated sample were observed during the storage. Enzymatic reaction could induce the conversion of carotenoid (main pigment for pineapple) which contributed to the color change. Carotenoid cleavage dioxygenases (CCD), lipoxygenase and peroxidase enzyme oversee breaking down the carotenoid molecules, which changes the color of the product (Hermanns et al., 2020).

Previous study on pineapple slices also found that HPP did not deactivate PPO or peroxidase (Woolf et al., 2013). Therefore, HPP could induce the color changes during the storage for ‘Phulae’ pineapple puree.

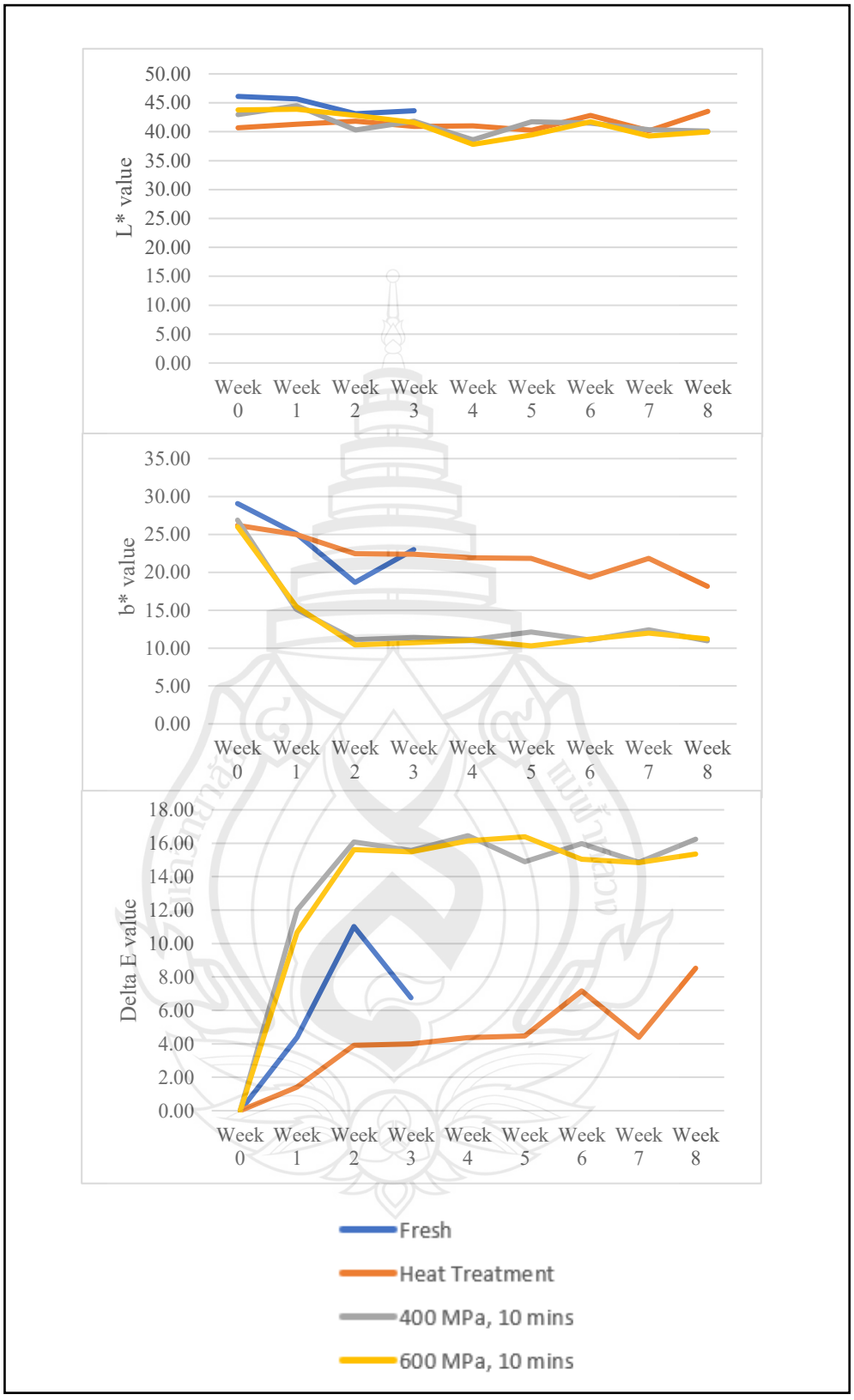


Figure 4.6 Color analysis of 'Phulae' pineapple puree during storage

Table 4.4 Physiochemical attributes of 'Phulae' pineapple puree during storage

Titratable Acidity (%)	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Fresh	0.63±0.01^{Bb}	0.62±0.03^{Bb}	0.70±0.01^{Ba}	0.69±0.02^{Ba}					
Heat Treatment	0.69±0.01^{Ab}	0.67±0.02^{Aa}	0.75±0.02^{Aa}	0.76±0.02^{Aa}	0.78±0.03^{Aa}	0.76±0.01^{Aa}	0.76±0.02^{Aa}	0.77±0.02^{Aa}	0.76±0.02^{Aa}
400 MPa, 10 mins	0.62±0.01^{Bb}	0.62±0.01^{Bb}	0.71±0.01^{Ba}	0.72±0.03^{Ba}	0.69±0.02^{Ba}	0.70±0.02^{Ba}	0.72±0.02^{Ba}	0.72±0.02^{Ba}	0.70±0.02^{Ba}
600 MPa, 10 mins	0.64±0.02^{Bc}	0.62±0.01^{Bc}	0.71±0.02^{Bb}	0.70±0.02^{Bb}	0.70±0.00^{Bb}	0.71±0.02^{Bb}	0.70±0.01^{Bb}	0.74±0.02^{ABa}	0.74±0.02^{Aba}
pH	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Fresh	3.91±0.00Aa	3.91±0.01ABa	3.90±0.01Aa	3.90±0.01Aa					
Heat Treatment	3.90±0.02Aab	3.92±0.00Aa	3.92±0.02Aa	3.90±0.01Ac	3.90±0.01Ac	3.89±0.02Acd	3.89±0.01Acd	3.87±0.00Ad	3.91±0.00Aab
400 MPa, 10 mins	3.91±0.01Aa	3.90±0.01Bab	3.90±0.01Aab	3.88±0.01Acd	3.89±0.01Abc	3.87±0.00Bde	3.84±0.01Bf	3.85±0.00Bef	3.89±0.00Bbc
600 MPa, 10 mins	3.91±0.00Aa	3.91±0.00ABa	3.90±0.00Aa	3.88±0.01Ab	3.89±0.00Ab	3.86±0.01Bc	3.84±0.01Bd	3.85±0.00Bcd	3.89±0.00Bb
Total Soluble Solid (°Brix)	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Fresh	11.69±0.05Ba	11.76±0.13Ba	11.76±0.05Ca	11.71±0.13Ba					
Heat Treatment	12.67±0.10Aab	12.64±0.22Aab	12.62±0.18Aab	12.61±0.39Aab	12.77±0.13Aa	12.34±0.16Ab	12.31±0.13Ab	12.44±0.21Aab	12.42±0.07Aab
400 MPa, 10 mins	11.70±0.03Bb	11.71±0.04Bb	11.97±0.07Ba	11.88±0.11Ba	11.93±0.03Ba	11.93±0.09Ba	11.93±0.06Ba	11.99±0.05Ba	11.93±0.03Ba
600 MPa, 10 mins	11.69±0.13Bd	11.77±0.12Bcd	11.94±0.05BCab	11.84±0.05Bbc	12.01±0.02Ba	11.92±0.04Bab	11.99±0.10Bab	11.93±0.09Bab	12.01±0.05Ba

Note Values are expressed as mean ± standard deviation. Different lowercase superscript letters within the same row indicate statistically significant differences during storage ($p < 0.05$), while different uppercase superscript letters within the same column indicate significant differences among treatments ($p < 0.05$)

4.3.1.2 pH

The pH of ‘Phulae’ pineapple puree are presented in Table 4.4. On week 0, there is no significant different in term of pH of all sample ($p<0.05$) which is between 3.90-3.91. However, during storage only fresh sample has no significant changes ($p<0.05$) over 3 weeks period of storage with pH range between 3.90-3.91. All treated sample (HT and HPP sample) have slight pH changes during the 8 weeks storage. However, it considered stable with pH range from 3.85-3.92. Despite HT samples shows higher pH during the storage, there is no significant different ($p<0.05$) between HT and HPP sample up to 4 weeks periods of storage. These results are consistent with the findings of Wu et al. (2021), who reported that 28 days of storage at 4 °C did not cause any significant changes in the pH of pineapple juice for either HT or HPP treated samples. After 5 weeks, HT pH is significantly higher compared to HPP treated samples. The lower pH on HPP treated sample are more likely due to the incomplete microbial inactivation which led into the growth of some microbes and/or the incomplete enzyme inactivation that could produce some organic acid (Bi et al., 2020; Suriati et al., 2020). Therefore, HT and HPP did not affect the pH of ‘Phulae’ pineapple puree in 4 weeks period.

4.3.1.3 Total Soluble Solid (TSS)

Total soluble solid of ‘Phulae’ pineapple puree are presented in Table 4.4. During the 8 weeks storage, the total soluble solid of all sample are around 11.69-12.77 with HT sample show the significant highest TSS among all samples ($p<0.05$). This probably due to the water reduction during the heat treatment, in which the water evaporated. There is no significant different in term of TSS between fresh and HPP samples (both 400 and 600 MPa for 10 mins) up to 3 weeks storage. This finding The slight increase in TSS on fresh and HPP treated sample may be attributed to the breakdown of complex sugars into simpler sugars during storage (Hussain et al., 2013). Therefore, HPP has no effect on total soluble solid of ‘Phulae’ pineapple puree.

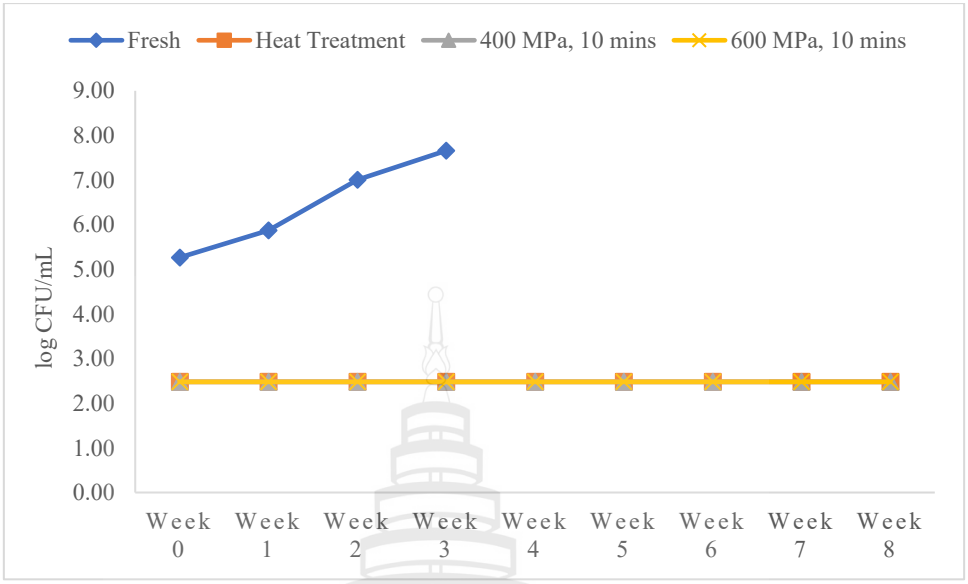
4.3.1.4 Titratable Acidity (TA)

Titratable acidity of ‘Phulae’ pineapple puree are presented in Table 4.4. Overall, the TA of all sample are around 0.62-0.78 with HT samples shows significant higher TA during the storage ($p<0.05$). All the samples have slight increase of TA after 2 weeks storage. This probably due to the breakdown of pectin substance into pectic acid or the formation of acidic compounds by the degradation and oxidation of reducing sugar (Awais et al., 2025). However, there is no significant difference between fresh and HPP treated sample in term of TA during storage ($p<0.05$). Therefore, HPP at 400 and 600 MPa for 10 mins has no effect on titratable acidity.

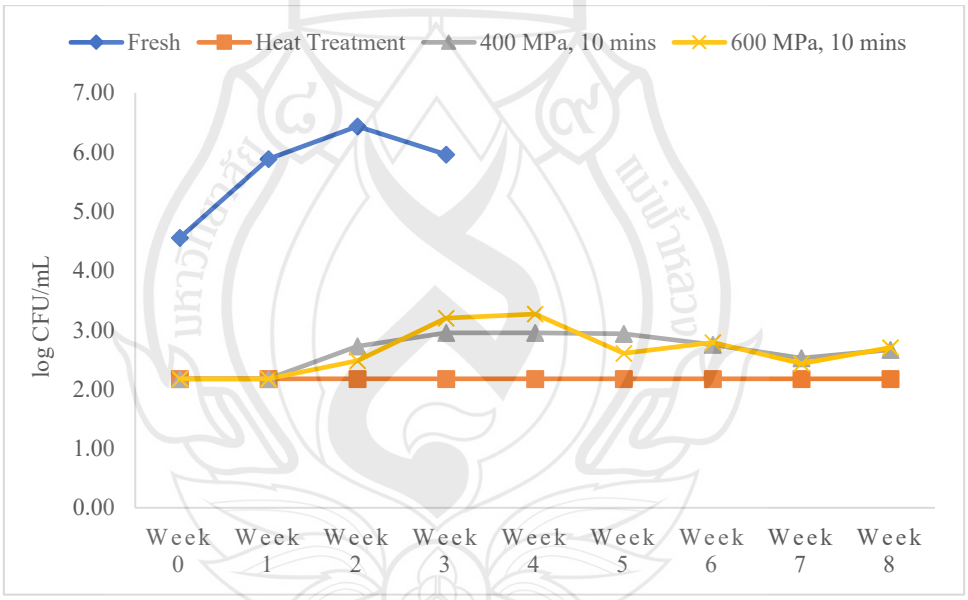
4.3.2 Quality Attributes: Microbial

The microbial analysis including aerobic plate count (APC) and yeast and mold (YM) for ‘Phulae’ pineapple could be seen in Figure 4.7. Fresh sample has higher APC and YM compare to the treated sample, which increase during the storage. This indicates that fresh sample have high microbial safety risk. The reason the observation stopped at week 3 for fresh sample is due to the high amount of microbes which resulted in the bloating of the samples package.

Despite the initial stable result of the HT and HPP with the microbial count decreased to less than 2.48±0.00 (APC) and 2.18±0.00 (YM) log CFU/mL, reducing the microbes by almost 2 log unit, only HT sample remains stable until the end of the 8 weeks storage. However, for HPP treated sample, both at 400 and 600 MPa, the YM count increases during the storage, but still less than 3.20±0.53 log CFU/mL. This is probably due to the incomplete inactivation of yeast and mold during the HPP treatment. There are various factors that could affect the inactivation of microbes by HPP, such as the magnitude of pressure and holing time, the process temperature, compression and decompression rate, the microbiota, and the intrinsic component of the product itself (Podolak et al., 2020). Higher pressure or longer period of treatment should be applied. Therefore, HPP could produce the safe ‘Phulae’ pineapple puree product with low load of microbes, similar to HT samples.



(a)



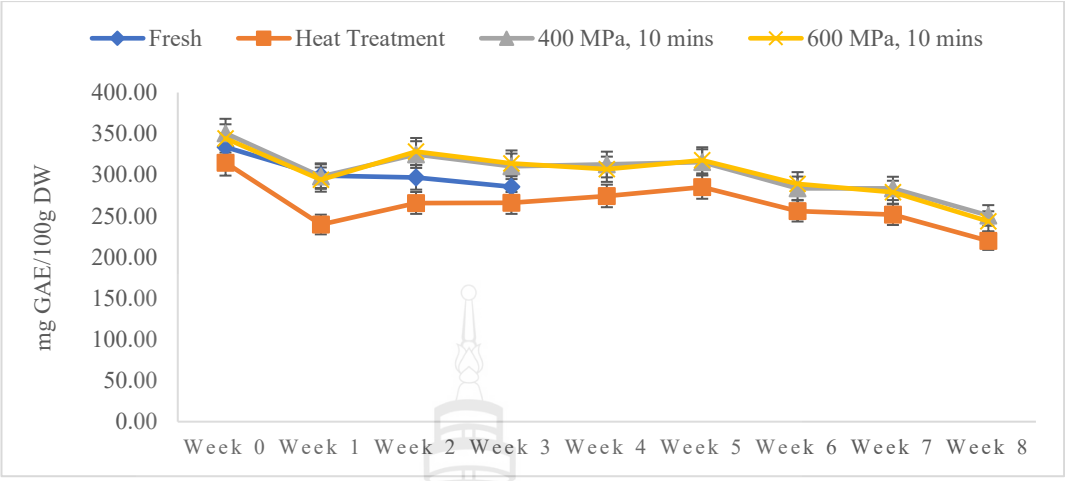
(b)

Figure 4.7 Aerobic plate count (a) and yeast and mold count (b) of 'Phulae' pineapple puree during storage

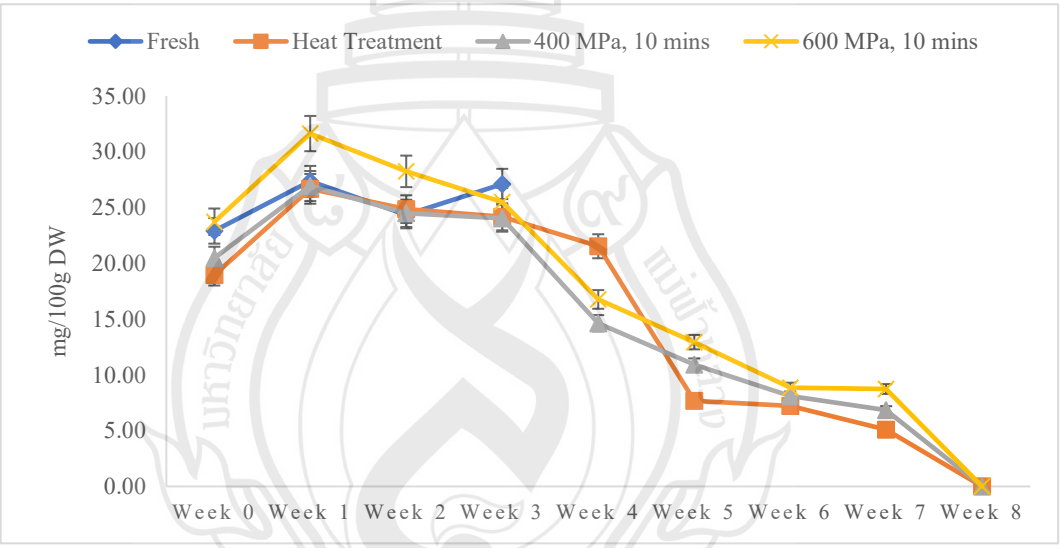
4.3.3 Bioactive Compounds: Total Phenolic Compound and Vitamin C

The total phenolic compounds (TPC) and vitamin C of 'Phulae' pineapple puree are presented in Figure 4.8. TPC of HPP treated sample at 400 and 600 MPa for 10 mins shows significantly higher ($p<0.05$) TPC compare to fresh and HT sample during the initial treatment and throughout the storage period. However, during the storage, the TPC for all samples are decreasing up to 30% compared with the initial amount. Same trend also observed on the vitamin C value. Despite the increase of the vitamin C on all the samples after 1 week storage, the value decrease during the storage. However, HPP treated samples still have significant higher vitamin C ($p<0.05$) compared to other samples.

The decrease of TPC during the storage for all sample is due to the degradation of phenolic compounds, which involved several pathways such as the phenol oxidations (autoxidation by metal ions and light), and the polymerization reaction between phenols (Teribia et al., 2021). The degradation also could be the result of the incomplete inactivation of polyphenol oxidase (PPO), an oxidoreductase-copper containing metalloprotein, which is a catalysator of phenolic compound degradation to o-quinones when the oxygen in present (Silva & Sulaiman, 2022). The oxygen present on the packaging is due to the trapped oxygen during the packaging process and oxygen permeability of the LLDPE packaging itself, which can still allow oxygen exchange, even in very small amount (Batista et al., 2022).



(a)

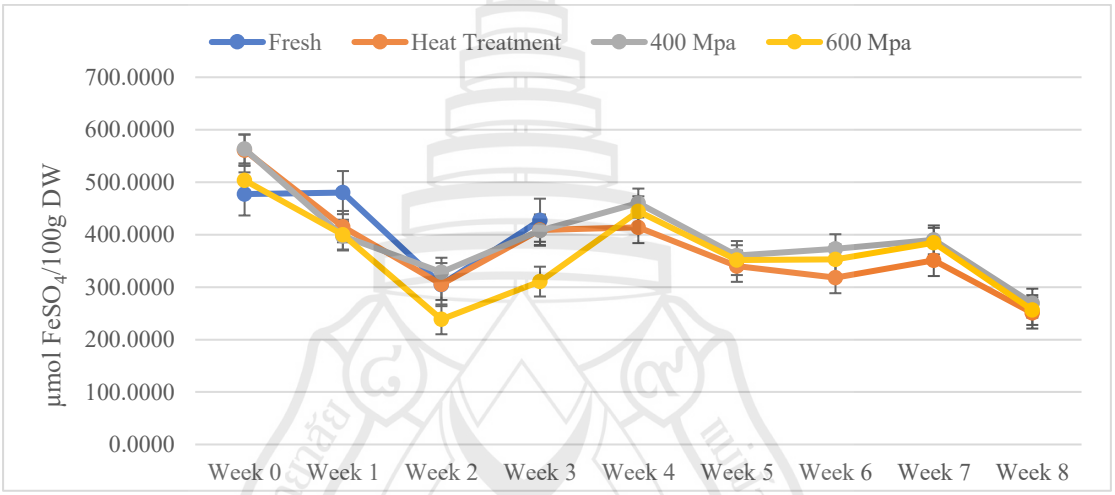


(b)

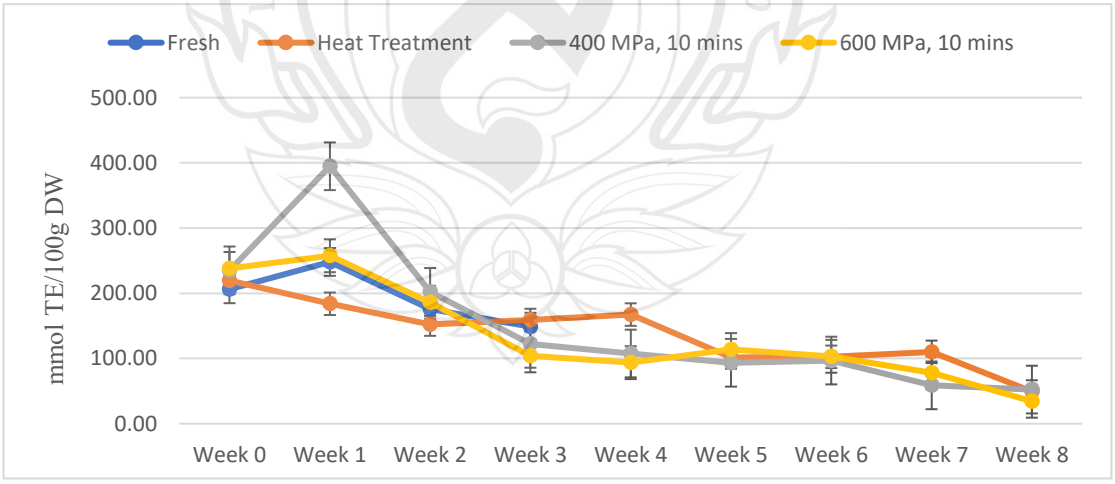
Figure 4.8 Total phenolic compound (a) and vitamin C content (b) of 'Phulae' pineapple puree during storage

4.3.4 Antioxidant Activity: DPPH and FRAP Capacity

Antioxidant activity of 'Phulae' pineapple puree are shown in form of DPPH and FRAP capacity in Figure 4.9. HPP treated samples shows higher DPPH and FRAP value during the initial storage. However, the DPPH and FRAP capacity of all samples shows similar trend which is decreasing during storage. The decrease of the antioxidant capacity of the puree are related to the bioactive compound content. Since the bioactive compound (TPC and vitamin C) of the samples also decreasing, the antioxidant activity will also follow the trend.



(a)

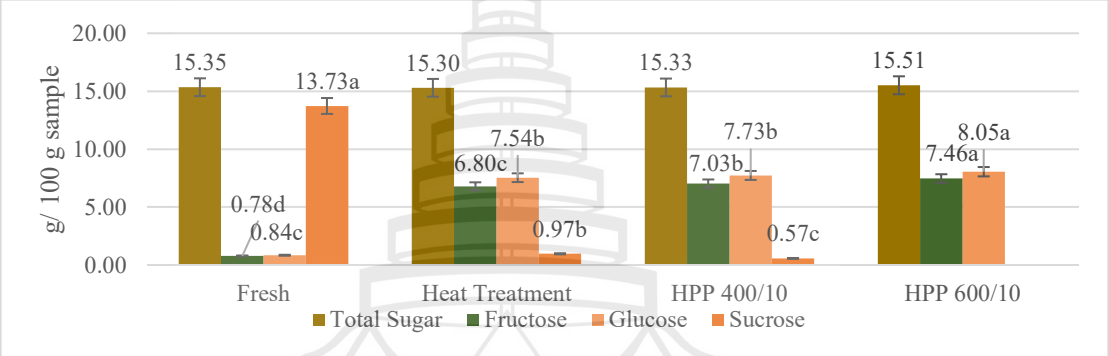


(b)

Figure 4.9 FRAP antioxidant capacity (a) and DPPH radical scavenging activity (b) of 'Phulae' pineapple puree during storage

4.4 Effect of HPP on ‘Phulae’ Pineapple Puree’s Glycemic Index

In these studies, the sample that analyzed were fresh sample, HT sample and the selected sample from the preliminary process, which is HPP at 400 and 600 MPa for 10 mins.



Note Data of different alphabets in the same color graph were different with statistical significance ($p<0.05$)

Figure 4.10 Total sugar and sugar content of ‘Phulae’ pineapple puree

As shown in Figure 4.10, the total sugar for all the sample is similar, around 15.30-15.51g/100g sample. However, the sugar profile composition is slightly different among samples (Figure 4.11). Fresh sample has high percentage of sucrose, and very low in both fructose and glucose, with ratio 1: 1.1: 17.7 (fructose : glucose : sucrose). Cordenunsi et al. (2010) also found higher sucrose ratio in Perola pineapple pulp and core, which is 1 : 1.4 : 9 and 1 : 1.2 : 6.4 (fructose : glucose : sucrose) respectively.

The HT sample has almost similar profile with the HPP treated sample in which shows that fructose and glucose are accountable for the total sugar, with glucose value are the highest, follow by fructose. However, only HT and HPP at 400 MPa has sucrose (lowest amount). During the processing, for both HT and HPP, most of the sucrose which is polysaccharides is more likely breaking down into monosaccharides (glucose and fructose) which resulted higher proportion on those two types of sugar. Higher pressure (600MPa) resulted in higher conversion from the sucrose to fructose and glucose.

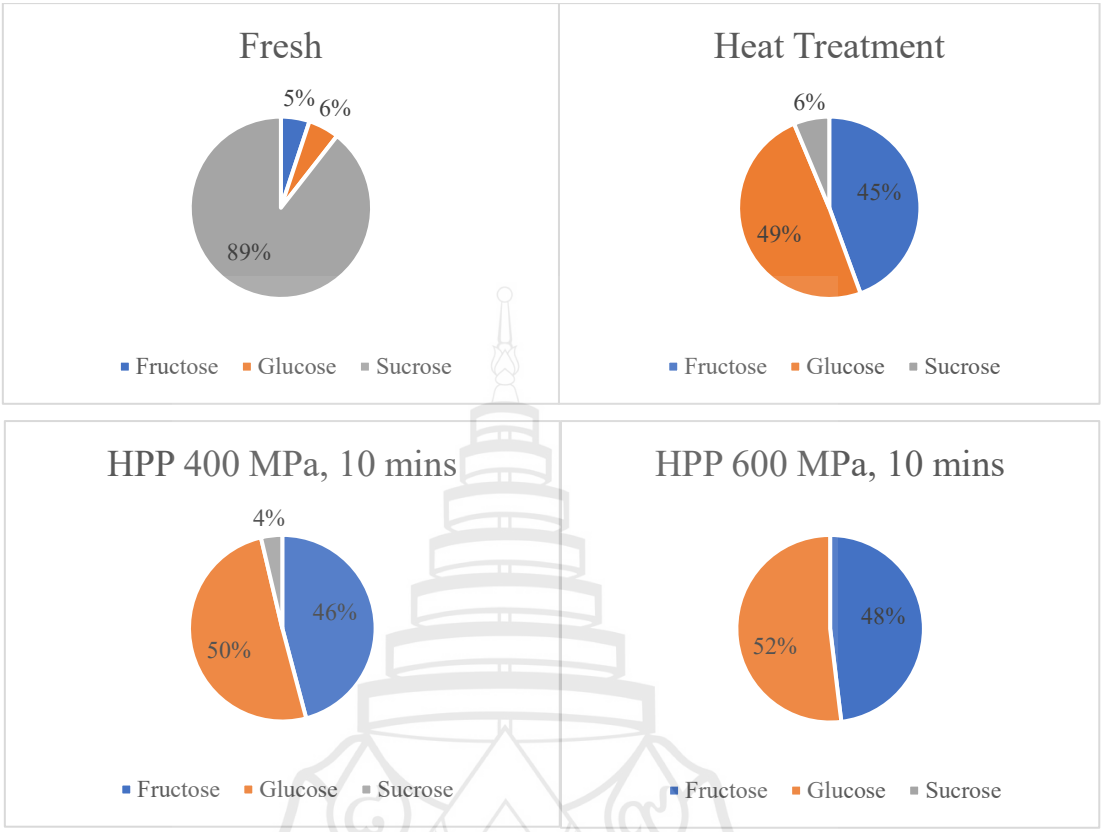
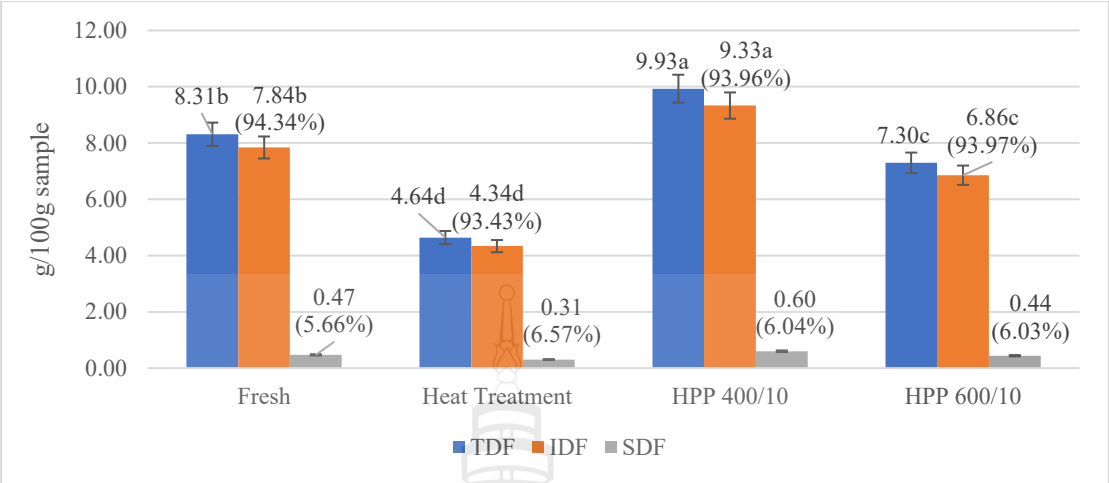


Figure 4.11 Sugar profile of 'Phulae' pineapple puree

Total dietary fiber (TDF) comprises Insoluble Dietary Fiber (IDF) and Soluble Dietary Fiber (SDF). In fresh and High-Pressure Processed (HPP) samples, the TDF ranges from 7.3 to 9.93 g per 100 g of the sample, whereas heat-treated samples have the lowest TDF at 4.64 g per 100 g. The lower TDF in HT sample is due to the breakdown of the fiber during the heat treatment. Higher TDF at lower pressure (400 MPa) is due to the higher fiber being extracted during the HPP treatment. However, higher treatment (600 MPa) has lower TDF compared to the fresh sample, is more likely due to the dietary fiber break down in high pressure.



***Note** Data of different alphabets in the same color graph were different with statistical significance ($p<0.05$)

Figure 4.12 Dietary fiber of 'Phulae' pineapple puree

The proportions of soluble and insoluble dietary fiber were varied as shown as in the percentage of the graph in Figure 4.12. In fresh samples, the soluble dietary fiber (SDF) is approximately 5.6%, similar with Perola pineapple, where the core and pulp have SDF contents of 5.8% and 2.5%, respectively (Cordenunsi et al., 2010). The fresh pineapple puree on these studies shows a slightly lower SDF proportion because it includes both the core and the pulp. After heat treatment (HT), the SDF increases to about 6.7%, and after HPP, it is around 6%. These changes are attributed to the inter-conversion of insoluble dietary fiber (IDF) to SDF during processing. Higher SDF indicates higher concentration of non-digestible oligosaccharides such as maltodextrins and other oligosaccharides which is the product of cell-wall breakdown (Elizondo-Montemayor et al., 2020).

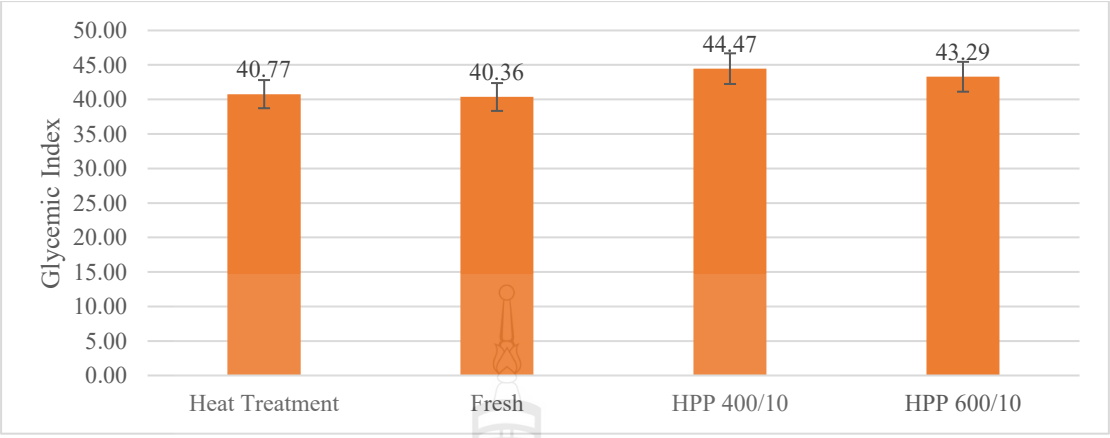


Figure 4.13 Glycemic Index of 'Phulae' pineapple puree

Glycemic index of all samples is between 40.36 to 44.47, with HT sample showing slightly higher value compared to the fresh sample while HPP treated sample has the highest value (400 MPa). The higher GI value in HPP treated sample could be due to the higher glucose in the sample (Figure 4.13). Despite the differences, the GI value for all sample, is still considered as low GI food (GI ≤ 55). Food considered has high GI if the GI ≥ 70 and medium GI with 56 ≤ GI ≥ 69 (Chou et al., 2020). The result showed lower value of GI compared to Perola pineapple pulp and core with GI value 93 and 95, respectively (Cordenunsi et al., 2010). However, this result contradicts previous findings, where HPP was shown to reduce the glycemic index (GI) in mango puree (HPP at 592 MPa for 3 mins, reducing GI from 42.7 to 32.7) and atemoya puree (HPP at 600 MPa for 15 mins, reducing GI from 64.5 to 49.8) as reported by Elizondo-Montemayor et al. (2015) and Chou et al. (2020). Consistent findings were reported by Wu et al. (2022), who observed that HPP at 600 MPa for 5 minutes reduced the GI of bananas from 85.1 to 50.3, while treatment at 600 MPa for 4.5 minutes lowered the GI of dragon fruit from 75.2 to 44.8.

4.5 Effect of HPP on ‘Phulae’ Pineapple Puree’s Anti–inflammatory Activity

Anti- inflammatory of the ‘Phulae’ pineapple puree are reflected through the nitric oxide (NO) inhibition. This study uses Trolox Equivalent (TE) to measure the inhibition effect on the samples. The initial treatment showed that both HT and HPP samples shows higher NO inhibition compared to fresh samples (1251.18-1575.44 mmol TE/100g DW), with HPP treated sample at 400 MPa shows the significant highest value. This shows similar result with study by Ke et al. (2021) on broccoli which mentions that the NO production on HPP treated broccoli was inhibited, which indicate the anti-inflammatory effect after the treatment.

During the storage, the amount decreased progressively and completely disappeared after 3 weeks of storage. This is indicated that even though ‘Phulae’ pineapple puree has anti-inflammatory effect, which most likely due to the high amount of its bioactive compound. However, the anti-inflammatory effect last less than the appearance of the bioactive compound itself, which remains in the samples (Figure 4.14). The inconsistency between the anti-inflammatory effect might be due to NO inhibition as the only analysis that used on this study. There is more method that could be used and other indicators to observe the anti-inflammatory effect, such as the hyaluronidase inhibitory activity.

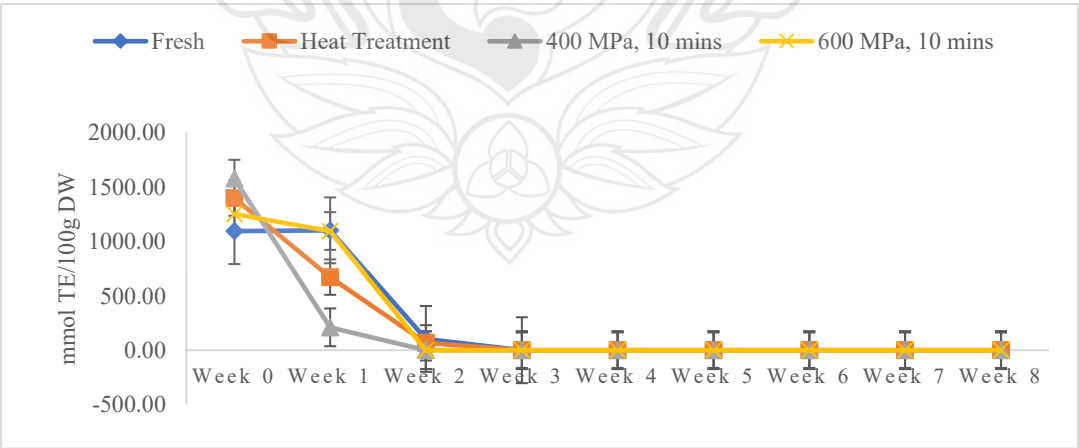


Figure 4.14 NO inhibition of ‘Phulae’ pineapple puree

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

'Phulae' pineapple puree that was subjected to HPP treatment (400 and 600 MPa, for 5, 10, and 15 mins) shows similar quality compared to the fresh sample, especially in physiochemical attributes, with higher pressure and longer processing time resulted in higher value of bioactive compounds and antioxidant capacity. Additionally, they also exhibit the same microbial reduction as the HT treated sample.

Fresh 'Phulae' pineapple puree only last for 3 weeks while the HT and HPP treated sample would last for 8 weeks. During storage, HPP and fresh sample showed the most color changes, but similar pH, TA, and TSS. Further analysis during storage shows that HPP at 400 and 600 MPa for 10 mins show incomplete inactivation of YM but fully eliminate the APC. The bioactive compound and antioxidant activity of all the samples were decreasing during storage.

HT and HPP treatment changed the sugar profile of the puree, with sucrose breaking down into glucose and fructose. This resulted in slightly higher GI on HPP treated sample compared to the fresh sample. HPP treated sample at 400 MPa for 10 mins shows the highest NO inhibition, which indicate the higher anti-inflammatory effect. Therefore, HPP treated 'Phulae' pineapple puree could be developed to be a new option for functional and healthier food.

HPP treatment at 400 MPa for 10 mins is the most optimal treatment for 'Phulae' pineapple product with similar effect to the HPP treated sample at 600 MPa for 10 mins, but higher NO inhibition effect. Besides, HPP at 400 MPa for 10 mins, will save more energy due to the lower pressure used for the processing.

5.2 Suggestions

In this study, the focused is only comparing between the heat treatment and high-pressure processing treatment towards the ‘Phulae’ pineapple puree. Even the result showed better nutritional and functional properties of the product through the application of HPP, further studies to optimize the application, to ensure complete microbial inactivation, especially yeast and mold, while preserving the desirable physiochemical (e.g. color) are still needed.

Further studies should also explore the application of hurdle technologies, combining the HPP with other preserving method to enhance the microbial safety without affecting the product quality. In addition, comprehensive sensory evaluation are recommended to assess consumer acceptance of color, flavor, texture, and overall preference of HPP-treated puree during storage. This will help determine market feasibility and consumer readiness for HPP-treated ‘Phulae’ pineapple puree as a functional food product.

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ACHIEVEMENT

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PUBLICATIONS

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